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THE INTERACTION OF MUTANTS AFFECTING VENATION IN DROSOPHILA MELANOGASTER. I. INTERACTION OF HAIRLESS, ENGRAILED AND CUBITUS INTERRUPTUS[1]

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ONE approach to the problem of how genes manifest themselves in development has involved the analysis of the effects of changes at a given gene locus, such changes taking the form of alterations in the combination, dosage and position of alleles. Except where the analysis of modifier action is involved, the emphasis of this type of study is on the effects of a disturbance in the normal genotypic complement at a single point. While this type of study has the advantage of dealing with relatively few genetic variables, it has the disadvantage of excluding from analysis the contributory action of the remainder of the genotype to the production of normality in the specific phenotypic character under investigation. The latter problem is perhaps better attacked by means of genetic interaction studies which make use of disturbances at several points in the genotypic system, analyzed singly and in combination.

Such genetic interaction studies have probably found greatest success in investigations dealing with the development of pigmentation in organisms. The work of WRIGHT (1941) on the interaction of the major color factors in the production of pigmentation in the coat of the guinea pig, the work of LAWRENCE and SCOTT-MONCRIEFF (1935) on gene interaction in the determination of flower color, and the work of BEADLE and EPHRUSSI (1936) on the genetic control of eye color in Drosophila are perhaps the most completely analyzed of such studies. Among other investigations primarily devoted to the study of gene interaction may be mentioned the work of NEEL (1941) and KARP (1936) on the interaction of loci concerned with bristle-forming reactions in Drosophila; that of CSIK (1934), SEMENZA (1951), and WALETSKY (1939) on the interaction of mutants affecting the Drosophila wing; the investigations of MAINX (1938) on gene interactions affecting eye color; the work of SEHULTZ (1939) on interactions dealing with the minute phenotype; and the study of CLARK (1949) on the interaction of loci affecting wing size in Habrobracon.

The system of veins in the Drosophila wing affords an unusually convenient phenotypic system for an interaction analysis. The relative simplicity of the pattern of longitudinal and cross veins, the precise manner in which the effects of various mutant substitutions on this pattern may be ascertained, and the large number of mutants available which produce disturbances in this pattern

[1] Adapted from a thesis submitted to the Graduate Division of the University of California at Berkeley in partial satisfaction of the requirements for the degree of Doctor of Philosophy.

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make the venation system an ideal point of departure for a quantitative study of interaction. Also, the availability of studies on normal and mutant development of the wing and veins (AUERBACH 1936; WADDINGTON 1940) makes possible an eventual extension of such an investigation to the developmental level.

The following report is the first in a contemplated series of researches dealing with the genetic control of morphogenesis of the vein system in *Drosophila melanogaster,* the present investigation being specifically concerned with the interaction of the cubitus interruptus, engrailed and Hairless mutants with respect to their interruption effects on the fourth longitudinal vein.

An account of the work to be presented has been published in abstract form (HOUSE 1952).

MATERIALS AND METHODS

The phenotypic point of departure for this study has been the fourth longitudinal vein in the wing of *Drosophila melanogaster,* in particular that portion extending distally from the posterior cross vein to the margin of the wing. Three mutants producing some degree of interruption of this vein have been chosen for investigation: the recessive mutant, cubitus interruptus (*ci,* fourth chromosome); the dominant mutant, Hairless (*H,* third chromosome); and the recessive mutant, engrailed (*en,* second chromosome).

Cubitus interruptus (TINIAKOV and TERENTIEVA 1933) causes an interruption of the fourth vein distal to and sometimes proximal to the posterior cross vein. The mode of action of this mutant has been rather extensively analyzed in a series of studies dealing with position effect (DUBININ and SIDOROFF 1934; STERN 1948; STERN, MACKNIGHT and KODANI 1946; STERN, SCHAEFFER and HEIDENTHAL 1946) and dosage relationships (STERN 1943). The latter work has been particularly useful in elucidating the general mode of action of alleles at this locus. The availability of the above-mentioned analysis makes this locus an especially useful one in interaction studies. The actual cubitus interruptus stocks employed in this study were cubitus interruptus (*ci/ci*) and cubitus interruptus, eyeless-Russian ($ci\ ey^R/ci\ ey^R$). Eyeless-Russian, which causes a variable but extreme reduction in the size of the eye, was used as a marker for the *ci* chromosome.

Hairless (BRIDGES and MORGAN 1923) is a homozygous lethal, dominant mutant. Its main effect is the removal of certain bristles, chiefly the postverticals, dorsocentrals, and abdominals. In addition there is generally an increase in eye size and an interruption of the fifth vein, and, under certain conditions, a loss of the most distal portion of the fourth vein. No detailed analysis of the action of this locus is available as in the case of cubitus interruptus, although its action in different dosages in triploids has been briefly investigated by GOWEN (1933). The experimental *H/+* stock used in the present study was extracted from a balanced *Pm/Cy; H/Sb* stock (*Sb,* Stubble; *Cy,* Curly; *Pm,* Plum).

The chief diagnostic character of engrailed is a splitting of the scutellum, ranging in degree from a flattening of the posterior scutellar edge to a deep

indentation (EKER 1929). In addition, the wings appear more truncate, with a variable interruption of the fourth vein at its distal tip. Some engrailed wings may show an added plexus of veins in the region of the fifth longitudinal vein. Engrailed also causes the production of an extra sex comb in males, the accessory comb lying on the medial side of the first tarsal joint and forming a mirror image of the normal comb. The mode of action of engrailed relative to the scutellar and sex comb characteristics has been analyzed by BRASTED (1941).

In addition to these three main mutants, the following stocks, normal and mutant, were employed in various stages of the work:

(1) The balanced stock, Minute-4/eyeless-dominant ($M\text{-}4/ey^D$). *M-4* (fourth chromosome) is a homozygous lethal dominant, deficient for the *ci* locus but not for that of ey^R, the salivary chromosomes showing an absence of several bands (BRIDGES 1935). Eyeless-dominant is a complex chromosome aberration in the fourth chromosome and affects the shape and size of the eyes.

(2) Eyeless-2 (ey^2), an allele of eyeless-Russian, with an effect on the eyes similar to that of ey^R.

(3) Canton Special (Can-S), a special wild-type stock (see STERN 1943).

(4) A balanced *Pm/Cy; H/Sb* stock from which the Hairless and Plum genes were extracted.

(5) The second chromosome homozygous lethal dominant Bristle (*Bl*), which produces a thickening and reduction in length of the bristles.

(6) The second chromosome minutes $M(2)I^2$ and $M(2)S7$.

(7) The third chromosome minutes *M(3)w and M(3)y.*

The M-4 stock was employed for the purpose of dosage studies, the second and third chromosome minutes were used to test the nature of the minute reaction with *en/en* and *H/+*, and the other stocks were utilized as markers only.

Crosses between the stocks previously mentioned furnished all stocks utilized in the study. No attempt was made to produce an isogenic background in the experimental stocks. Wherever possible, the crosses have been designed so that the genotypes to be compared occur as sibs from each of a series of replicated crosses.

All matings were made in half-pint milk bottles, employing the standard cornmeal-molasses-agar food medium, enriched with yeast and sown with a few drops of live yeast just prior to the introduction of the parent flies. Single pair matings were employed in all crosses. Females were allowed to lay eggs for seven or eight days prior to removal, counts being made for the first nine days of emergence. All crosses, unless otherwise noted, developed at $26 \pm 0.5°C$.

The degree of phenotypic expression of the various gene combinations was determined by measuring the extent of interruption of the fourth vein in the region distal to the posterior cross vein. For this purpose, following STERN (1943), five arbitrary classes were designated and the number of individuals falling into these classes was scored with respect to sex for the different genotypes of the various experiments. The class designations are as follows (fig. 1):

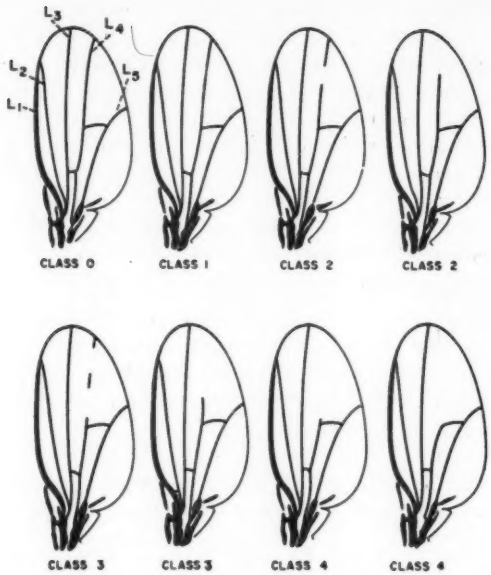

FIGURE 1.—Classification of the degree of interruption of the fourth vein.

Class 0—normal 4th vein.

Class 1—a distinct thinning of the 4th vein in one or more regions.

Class 2—one or more complete interruptions of the 4th vein, the sum of the lengths of the interruptions equaling less than one-half of the whole section between posterior cross vein and wing margin.

Class 3—one or more complete interruptions of the 4th vein, the sum of the lengths of the interruptions equaling more than one-half of the whole section.

Class 4—complete absence of the 4th vein between cross vein and wing margin, or the presence of a very short section projecting beyond the cross vein, or of a dot-like distal remnant of the vein not exceeding in length the width of the normal vein.

Both wings were examined wherever possible, and the degree of expression was recorded as an average of the two conditions.

RESULTS

The effect of ci, en and H and their combinations on the fourth vein

Table 1 summarizes the results of the following four crosses: (1) $+/+ \times H/+$ (2) $en/en \times en/en; H/+$ (3) $ci/ci \times H/+; ci/ci$ (4) $en/en; ci/ci \times en/en; H/+; ci/ci$. The distributions and comparisons of table 1 indicate in general a cumulative action of *en*, $H/+$ and ci/ci in the various combination genotypes. A clearer picture of this cumulative action can perhaps be obtained from a

TABLE 1

The interactions of engrailed, Hairless, and cubitus interruptus relative to their effects on the fourth longitudinal vein.[1]

Genotype	Females					Males					♀♀ vs. ♂♂[2]
	0	1	2	3	4	0	1	2	3	4	
(1) +/+	237	....	....	....	....	256	....	....	....	....	
(2) *H/+*	78	5	162	....	....	196	3	44	....	....	**
(3) *en/en*	25	9	35	....	....	28	15	38	....	....	#
(4) *en/en; H/+*	....	....	6	61	....	....	....	11	64	....	#
(5) *ci/ci*	43	10	144	25	....	26	13	123	51	....	**
(6) *H/+; ci/ci*	....	....	3	155	81	....	....	8	160	83	#
(7) *en/en; ci/ci*	....	....	....	53	76	....	....	....	39	89	#
(8) *en/en; H/+; ci/ci*	....	....	....	11	190	....	....	....	10	197	#

[1] **The following comparisons show a significant difference at the 1% level in both sexes: 3 vs. 4, 2 vs. 4, 5 vs. 6, 2 vs. 6, 5 vs. 7, 3 vs. 7, 7 vs. 8, 6 vs. 8, 4 vs. 8.**
[2] **# Not significant; * Significant at the 5% level; ** Significant at the 1% level.**

graphical representation of these interactions (fig. 2). For the presentation in figure 2, an average percentage of the amount of L_4 present relative to complete presence of the vein was computed for each genotype and sex of table 1, using as a basis the following transformation from expressivity grades to percentage values: Class 0–1, 100%; Class 2, 50 to 100%, midpoint, 75%; Class 3, 0 to 50%, midpoint 25%; Class 4, 0%. The midpoint percentage value for each class was multiplied by the number of individuals in that class, and a mean percentage figure determined for the distribution of each genotype. These

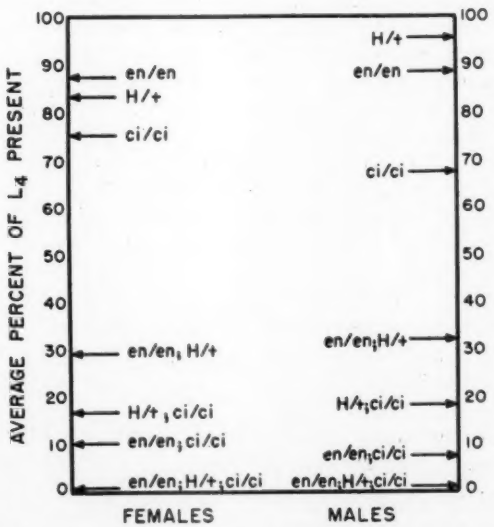

FIGURE 2.—Average percentage of L_4 present in *en/en*, *ci/ci*, *H/+* and their combinations.

mean values are indicated for males and females by the arrows of figure 2. For the present we may confine our attention to two aspects of the relationships indicated in figure 2. Perhaps the most interesting aspect relates to the fact that the degree of interruption of L_4 in any of the combination genotypes is considerably in excess of that expected by merely summating the interruption effects of the mutants considered singly. For example, on the average, *en/en* females cause a loss of 12.5% of the fourth vein, *H/+* females, a loss of 17%. Yet the *en/en; H/+* genotype in females brings about a loss of 71% of the fourth vein, in contrast to the expected 29.5% loss if the actions of the two mutants were strictly additive in combination. The second point of interest relates to the significant difference that exists between the sexes for the *ci/ci* and *H/+* distributions, but which is not reflected in the comparison of males and females of the *en/en; H/+, H/+; ci/ci, en/en; ci/ci* and *en/en; H/+; ci/ci* genotypes (table 1).

In addition to the question of the average amount of L_4 venation present in a particular genotype, there is also the question of the pattern of interruption which has produced this average. Although *H/+* and *ci/ci* females may bring about a similar average degree of interruption in L_4, the patterns by which this is accomplished are considerably different for the two genotypes. The question of the pattern of effect of *en/en, ci/ci, H/+* and their combinations will be treated in a subsequent paper.

Pleiotropic effects of ci, en and H and their combinations

While the chief concern of the present study is the nature of the interaction of *en, ci* and *H* relative to their effects on the fourth vein, brief mention should be made of the effects of these mutants on other parts of the venation system as well as on other structures of the adult fly. A more extensive analysis of this problem of pleiotropism will be reported separately.

Although no exact quantitative studies have been made, the engrailed characters extra sex comb (males) and cleft scutellum appear to be manifested in *en/en; H/+* flies to approximately the same degree as in *en/en*. This is not true, however, for *en/en; ci/ci*. In flies of the latter genotype there is a decided inhibition of these effects. In *en/en; ci/ci* males the extra sex comb may be completely missing or the number of teeth considerably reduced in number. In addition both *en/en; ci/ci* males and females show interference with the scutellar characteristic of engrailed. The scutellar cleft occurring in engrailed flies, while variable in respect to the depth of the cleft, is usually bilaterally symmetrical. In the presence of cubitus interruptus the scutellum loses its symmetry about the cleft, the latter in some cases being completely obliterated. The above-mentioned inhibition of pleiotropic effects of engrailed in *en/en; ci/ci* is found also in *en/en; H/+; ci/ci* flies.

In addition to the effects on L_4, other parts of the vein system may show interruptions when *en, ci* and *H* are combined. To a varying degree interruptions may be found in the distal portion of L_2, in both middle and terminal regions of L_3, in L_5 both proximal and distal to the posterior cross vein, and

TABLE 2

Venation effects other than interruption of L_4 of en/en, ci/ci, H/+ and their combinations.

Genotype	Percentage[1] of Interruption of											
	L_3		L_5		L_5 proximal to posterior cross vein		L_5 distal to posterior cross vein		L_4 proximal to enterior cross vein		Anterior cross vein	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
en/en	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
H/+	0.0	....	0.0	....	0.0	....	79.5	....	0.0	....	0.0	....
en/en; H/+	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0
ci/ci	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	5.5
H/+; ci/ci	0.5	2.5	3.0	4.5	2.5	1.5	100.0	100.0	0.0	0.0	7.5	6.5
en/en; ci/ci	1.0	1.0	0.0	0.0	35.5	43.0	11.0	15.0	67.5	57.0	8.5	14.0
en/en; H/+; ci/ci	77.5	91.0	15.5	12.0	72.5	74.0	100.0	100.0	73.5	68.5	27.0	21.5

[1]Percentage determinations based on right and left wings combined; *en/en* counts based on 60 wings; *en/en; H/+* counts based on 100 wings; counts for all other genotypes based on 200 wings.

in L_4 proximal to the anterior cross vein. In addition to these effects, the anterior cross vein may be missing in part or entirety. With the exception of the interruption of the distal portion of L_5, characteristic of Hairless, and the low penetrance of interrupted anterior cross veins in *ci/ci* individuals, these other venation characteristics appear as new effects in the combination genotypes (table 2). With reference to these new characteristics of venation there seems to be the same type of augmentation of effect when single mutants are combined as was demonstrated for interruption of L_4.

Changes in dosage at the ci locus in the presence of the en and H substitutions

STERN (1943) has demonstrated that greater normality of L_4 venation results with an increase from one to two doses of the *ci* allele in diplo-IV flies. The question is thus raised whether this increase in normality of the fourth vein with increasing dosage of the *ci* allele also takes place in the presence of the *en* and *H* substitutions. To answer this question relative to *H* the following cross was made: $H/+; ci\ ey^R/ci\ ey^R \times ci\ ey^R/M\text{-}4$. The results of this cross (table 3) bear out the dosage relationship established for *ci* by MOHR (1933)

TABLE 3

The effect of increase in dosage of the ci allele in the presence and absence of the Hairless substitution.[1]

Genotype	Females					Males				
	0	1	2	3	4	0	1	2	3	4
(1) $ci\ ey^R/ci\ ey^R$	32	49	162	55	....	13	22	134	58	....
(2) $ci\ ey^R/M\text{-}4$	1	....	14	41	10	2	2	11	70	26
(3) $H/+;\ ci\ ey^R/ci\ ey^R$	....	1	11	151	63	....	....	13	166	110
(4) $H/+;\ ci\ ey^R/M\text{-}4$	1	1	....	1	13	1	....	4	4	29

[1]The following comparisons show a significant difference at the 1% level in both sexes; 1 vs. 2, 1 vs. 3, 2 vs. 4, 3 vs. 4.

and STERN (1943). The increase in normality with the increase in dosage at the *ci* locus also takes place in the presence of the Hairless substitution, as the comparison of *H/+; ci ey^R/ci ey^R* with *H/+; ci ey^R/M-4* flies indicates. The question whether the Minute-4 normal eye and the non-minute eyeless-Russian phenotypes, which characterize the single dose and double dose genotypes respectively, act as modifiers to cause *M-4/ci ey^R* flies to be more extreme than *ci ey^R/ci ey^R* regardless of dosage relationships, has been answered in the negative by STERN (1943). To test the possible differential modifying effects of the *ey^R/ey^R* and *ey^R/+* combinations which characterize the double and single dose conditions, respectively, in the Hairless cubitus interruptus combination, the following cross was made: *H/+; ci ey^R/ci ey^R* × *ci ey^R/ci+* (table 4). As STERN found, there is no significant difference between the *ci ey^R/ci ey^R* and *ci ey^R/ci+* genotypes for either males or females. The same is shown here to be true with respect to the *H/+; ci ey^R/ci ey^R* vs. *H/+; ci ey^R/ci+* comparison. From this we may conclude that whatever the effect of eyeless-Russian on the action of *ci* and *H* may be, it is of the same magnitude in both the homozygous and heterozygous conditions. No information is available on the nature of the dosage response at the *ci* locus in the presence of the engrailed substitution, since repeated crosses of the type *en/en; M-4/+* × *en/en; ci ey^R/ci ey^R* have failed to yield any engrailed Minute-4 flies. Apparently the *en/en; M-4/ci ey^R* combination is lethal.

TABLE 4

***The modifying role of the eyeless-Russian phenotype on ci L$_4$ interruption in the presence and absence of the Hairless substitution.*[1]**

Genotype	Females					Males				
	0	1	2	3	4	0	1	2	3	4
ci ey^R/ci ey^R	9	....	122	82	....	7	1	137	102	....
ci ey^R/ci +	11	3	200	123	....	11	3	173	102	....
H/+; ci ey^R/ci ey^R	....	....	5	183	22	....	....	5	191	22
H/+; ci ey^R/ci +	....	....	4	278	32	....	....	10	259	24

[1] **The following comparisons show no significant difference in either sex: *ci ey^R/ci ey^R* vs. *ci ey^R/ci +*, *H/+; ci ey^R/ci ey^R* vs. *H/+; ci ey^R/ci +*.**

STERN and SCHAEFFER (1943) have reported an interruption of L$_4$ in hemizygotes of different wild type *ci* alleles at low temperatures (14°). This effect is more extreme in the hemizygotes than in the corresponding homozygotes at the same temperature, the latter exhibiting practically normal venation at 14°. These results might suggest that one dose of the normal *ci* allele is not as successful in elaborating some precursor material necessary for vein formation as two doses are, and that low temperature shows up these differences which remain subthreshold at the higher temperature levels (at 26° *ci$^+$/M-4* flies show normal venation).

The question then suggests itself whether possible subthreshold differences in the activities of the *ci$^+$/M-4* and *ci$^+$/ci$^+$* genotypes at higher temperatures might be brought out by interaction with *H/+* and *en/en*. To test this possi-

bility with respect to *en/en* the following cross was made: *en/en; M-4/+* × *en/en* (table 5). The results indicate a greater abnormality of L_4 venation in *en/en; M-4/+* than in *en/en*. We are faced with two possible interpretations of the interaction of *en/en* and *M-4/+* relative to their effect on the fourth vein. It may be that this represents a true dosage effect and that the combination of subthreshold deficiency in vein material due to one dose of ci^+ and the action of *en/en* produces cumulative results, with a resulting shift in venation away from normality. Or it is possible that *M-4* acts in some way as an intensifier of *en/en*, this action in no way being related to the single dose of ci^+. The second possibility would perhaps not be expected, due to the general intensifying effect of Minutes on extra-venation mutants such as net and plexus (*px*) (see BRIDGES and BREHME 1944).

A decision between these two alternative explanations can perhaps be made by using Minutes other than *M-4*, inasmuch as Minutes in general exhibit the same complex of effects on Drosophila development, producing deviations from

TABLE 5

The interaction of engrailed with various minutes relative to L_4 interruption.[1]

Genotype	Females					Males				
	0	1	2	3	4	0	1	2	3	4
(1) *en/en*	10	8	271	....	....	8	10	230	1	....
(2) *en/en; M-4/+*	26	10	105	25	2	9	6	116	63	3
(3) *en/en*	36	13	331	3	....	55	33	311	6	....
(4) *en/en; M(3)w/+*	65	1	14	1	....	78	12	57	1	....
(5) *en/en*	19	11	169	....	....	48	26	82	....	....
(6) *en/en; M(3)y/+*	14	....	....	....	....	37	3	9	....	....

[1] **The following comparisons show a significant difference at the 1% level in both sexes: 1 vs. 2, 3 vs. 4, 5 vs. 6.**

normality in the eye, bristles, developmental time, etc. The interaction of *en/en* with other Minutes presents a Minute-engrailed interaction minus the dosage effect at the *ci* locus.

To settle this issue the following crosses were made, utilizing two third chromosome minutes, *M(3)w* and *M(3)y*: *en/en; M(3)w/+* × *en/en* and *en/en; M(3)y/+* × *en/en*. The results of these crosses are presented in table 5. The *en-M(3)w* and *en-M(3)y* flies are relatively inviable, showing a considerable reduction in wing size, with wings in some flies appearing dumpy-like and exhibiting a certain amount of blistering. These wing abnormalities were not observed in the *en/en; M-4/+* genotype.

The results of the above crosses show a significant inhibition of the action of *en/en* rather than the enhancement to be expected if the increased interruption in *en/en; M-4/+* were the result of a general Minute action rather than a consequence of the single dose of the normal *ci* allele. While these results may suggest that the increased degree of interruption in *en/en; M-4/+* is due to the single dose of the normal *ci* allele, there still exists the possibility that the general manner of interaction of *M-4* and *en* on the fourth vein is different

from that of *M(3)w* and *M(3)y* with *en*, considering only the Minute effects themselves. The difference in wing size and shape between *en/en; M-4/+*, on the one hand, and *en/en; M(3)w/+* and *en/en; M(3)y/+*, on the other hand, certainly suggest caution before ruling out the latter possibility. If the increased interruption of L_4 in *en/en; M-4/+* is due to the hemizygous ci^+ condition, then we might suspect that this enhancement is greater than that indicated by the *en/en* vs. *en/en; M-4/+* comparison, part of the action being counteracted by an inhibition of *en/en* by *M-4* similar to that found in *en/en; M(3)w/+* and *en/en; M(3)y/+*. It would be desirable to test the interaction of *en/en* with a wider range of Minutes than the two used. On the other hand, a further indication that the enhancement of L_4 interruption found in *en/en; M-4/+* is due to a subthreshold effect at the *ci* locus is the observation that the pleiotropic engrailed effects inhibited in *en/en; ci/ci* are also inhibited in *en/en; M-4/+*.

TABLE 6

The interaction of Hairless with various minutes relative to L_4 interruption.[1]

Genotype	Females					Males				
	0	1	2	3	4	0	1	2	3	4
+/+	332	....	....	....	....	371	....	....	....	....
M-4/+	170	....	....	....	....	214	....	....	....	....
H/+	226	12	92	....	....	269	2	15	....	....
H/+ M-4/+	167	....	4	....	....	249	1	4	....	....
+/+	316	....	....	....	....	304	....	....	....	....
$M(2)l^2$/+	168	....	....	....	....	129	....	....	....	....
H/+	136	7	109	....	....	183	3	38	....	....
$M(2)l^2$/+; H/+	132	1	9	....	....	184	1	....	....	....
+/+	347	....	....	....	....	316	....	....	....	....
M(2)S7/+	220	....	....	....	....	230	....	....	....	....
H/+	111	2	148	....	....	241	10	42	....	....
M(2)S7/+; H/+	110	1	17	....	....	142	....	5	....	....

[1] **The following comparisons show a significant difference at either the 1% or 5% level in both sexes: *H/+* vs. *H/+; M-4/+*, *H/+* vs. *M(2)l^2/+; H/+*, *H/+* vs. *M(2)S7/+; H/+*.**

The interaction of *H/+* with hemizygous ci^+ was tested by means of the following cross: *H/+* × *M-4/+*. The results are given in table 6. Although the degree of interruption of L_4 in *H/+* males and females is not as great as that previously found for this genotype (table 1), a significant inhibition of the *H/+* action is seen in *H/+; M-4/+* females, the shift toward normality in *H/+; M-4/+* males, compared to *H/+*, being of doubtful significance. Again we are faced with the question whether the indicated inhibition of *H/+* in *H/+; M-4/+* individuals is due to the hemizygous ci^+ condition, to the presence of the Minute effects themselves aside from dosage considerations, or to a combination of both. To answer this question in part we may, as in the case of *en/en*, make use of the interaction of *H/+* with other Minutes.

The results of crosses between *H/+* and two second chromosome Minutes, *M(2)-l^2* and *M(2)-S7*, are presented in table 6. The comparisons, *H/+* vs.

M(2)-I²/+; H/+ and *H/+* vs. *M(2)-S7/+; H/+* reveal the same significant inhibition of *H/+* as was demonstrated for *M-4/+*. This would suggest that the increasing normality of venation in *H/+; M-4/+* is a result of the inhibitory Minute action and is not attributable to the hemizygous condition of the ci^+ allele. It is possible, therefore, that the action of *H/+* in interrupting L_4 is increased in the presence of *M-4* as was the case for *en/en*, but that the effect is not great enough to counteract the general enhancement by Minutes of extra venation. As in the case of engrailed, it would be desirable to test the interaction of *H/+* with a larger spectrum of Minute types.

The action of engrailed and cubitus interruptus heterozygotes

Previous results have indicated a considerable enhancement of the venation effects in the various combinations of *en, ci* and *H* over that expected on the basis of a simple summation of the measurable effects found in the single mutant genotypes. These "super-additive" results (NEEL 1941) might be interpreted on a basis of cumulative action of subthreshold effects of the single mutants, such action in the combination genotypes making possible the transgression of thresholds and the bringing of unsuspected mutant attributes into measurable range. This type of interpretation of the interaction effects of *en, H* and *ci* will be analyzed in considerable detail in a later paper. In view of these considerations, it seemed desirable to test the effect of *en* and *ci* heterozygotes on *ci* and *en* homozygotes, respectively, as well as on *H/+*, the thought being that while *en/+* and *ci/+* show completely normal venation at 26°, these genotypes may actually differ at the subthreshold level from the corresponding homozygous normal genotypes, en^+/en^+ and ci^+/ci^+. To test the above possibility the series of crosses indicated in tables 7, 8, 9 were carried out.

The results of the cross, *en/en; ci ey^R/ci ey^R* × *en/+; ci ey^R/++* (table 7), indicate an enhancement of *en/en* and *ci/ci* interruption by *ci/+* and *en/+*, respectively. The pattern of interruption found in the *en/en; ci ey^R/++* geno-

TABLE 7

The interaction of engrailed and cubitus interruptus in homozygous and heterozygous combinations.[1]

Genotype	Females					Males				
	0	1	2	3	4	0	1	2	3	4
(1) *en/+; ci ey^R/++*	231	....	....	....	....	215	....	....	....	....
(2) *en/+; ci ey^R/ci ey^R*	23	14	92	33	1	6	6	91	64	5
(3) *en/en; ci ey^R/ci ey^R*	....	....	....	35	95	....	....	3	21	79
(4) *en/en; ci ey^R/++*	14	26	133	28	3	10	12	103	67	10
(5) *Bl/+; ci ey^R/ci ey^R*	43	22	204	48	....	6	5	148	121	....
(6) *en/+; ci ey^R/ci ey^R*	20	12	222	89	....	3	1	101	202	1
(7) *Bl/+; ci ey^R/ci ey^R*	49	25	218	32	....	19	11	192	43	....
(8) *ci ey^R/ci ey^R*	36	13	212	27	....	17	6	250	52	....

[1] **The following comparisons show a significant difference at the 1% level in both sexes: 2 vs. 3; 3 vs. 4; 5 vs. 6. The comparison, 7 vs. 8, shows no significant difference in either sex.**

type is completely different from the solely terminal interruption characteristic of the *en/en* genotype (table 1) and now resembles the pattern characteristic of *ci/ci*. A χ^2 comparison of the *en/en; ci ey^R/++* distribution of table 7 with the *en/en* distribution of table 1 shows a significant difference for both males and females at the one percent level. In light of the apparent superposition of the *ci* pattern of L_4 interruption on that for *en* in the *en/en; ci ey^R/++* genotype, it seems safe to conclude that *ci/+* brings about an enhancement of the *en/en* interruption effect, even though the actual genotypes compared result from crosses performed at different times and may represent different genic backgrounds.

A comparison of *en/+; ci ey^R/ci ey^R* flies (table 7) with *ci ey^R/ci ey^R* (table 3) shows a significant shift (one percent level) toward a greater degree of interruption in *en/+; ci ey^R/ci ey^R* flies as compared to *ci ey^R/ci ey^R* for males, but the slight shift in the same direction for females is not significant. However, these genotypes have been compared on a basis of progeny resulting from crosses performed at different times and not segregating within the same bottle and genic environments. In view of this consideration, and the fact that there is no radical change in the pattern of interruption of *en/+; ci ey^R/ci ey^R* as compared to *ci ey^R/ci ey^R* (as was the case for the *en/en* vs. *en/en; ci ey^R/++* comparison), it seemed desirable to investigate further the effect of *en/+* on *ci/ci*.

To do this, the following cross was made (table 7): *ci ey^R/ci ey^R* × *Bl+/+en; ci ey^R/ci ey^R*. This cross makes possible a comparison between *ci ey^R/ci ey^R* and *en/+; ci ey^R/ci ey^R* where these genotypes occur as segregants in the same bottle. The results indicate a significantly more abnormal venation in the *en/+; ci ey^R/ci ey^R* flies than in the *Bl/+; ci ey^R/ci ey^R* flies, for both males and females. There still remains the possibility, however, that the marker gene Bristle exerts an inhibitory effect on *ci* interruption of a degree sufficient to produce the difference observed between the two genotypes under consideration. This possibility can be tested by the cross: *ci ey^R/ci ey^R* × *Bl/+; ci ey^R*. The results (table 7) show no significant difference between these two genotypes in either sex. In the light of the above results, it is possible to conclude that there is a significant enhancement of L_4 interruption in *ci ey^R/ci ey^R* by *en/+*.

To test the interaction of Hairless with heterozygous cubitus interruptus a cross was set up to furnish *H/+* and *H/+; ci ey^R/++* as segregating genotypes in each of a set of culture bottles. This was accomplished by making the following cross: *H/+* × *ci ey^R/+ ey^D*. The results of this cross are given in table 8, top portion.

With the exception of a single fly of the *H/+; ci ey^R/++* genotype, all flies regardless of genotype exhibited normal venation. Apparently *ey^D* and either *ci/+*, *ey^R/+* or their combination has suppressed the action of *H* on the fourth vein.

A second attempt was made to compare *H/+* and *H/+; ci ey^R/++* by making the cross *H/+; ci ey^R/++* × *ey^2/ey^2* (table 8, second part). The results indicate inhibition of *H* action by heterozygous *ey^2* as well as by *ey^2/ey^R*.

To clarify further the manner of interaction of $H/+$ with $ci\ ey^R/++$ the following crosses were made at 30°, all bottles occupying the same incubator shelf: (1) $H/+ \times +/+$ (2) $H/+ \times ey^2/ey^2$ (3) $H/+ \times ci/ci$ (4) $H/+ \times ci\ ey^R/ci\ ey^R$. Unfortunately all bottles of the $H/+ \times ci/ci$ and $H/+ \times ci\ ey^R/ci\ ey^R$ crosses were sterile. The results of the other two crosses (table 8) show a significant suppression of the action of $H/+$ by $ey^2/+$ for both males and females, as was the case at 26°.

Finally, the following crosses were made at 26°, the culture bottles residing on the same incubator shelf: $H/+ \times +/+$ and $H/+ \times ci/ci$. The results (table 8) indicate no significant difference between the distributions for $H/+$ and

TABLE 8

The interaction of Hairless with heterozygous cubitus interruptus.†

Genotype	Females					Males				
	0	1	2	3	4	0	1	2	3	4
$H/+;\ ci\ ey^R/++$	216	....	....	....	....	211	....	1	....	....
$H/+;\ ey^D/+$	194	....	....	....	....	214	....	....	....	....
$ci\ ey^R/++$	202	....	....	....	....	183	....	....	....	....
$ey^D/+$	225	....	....	....	....	216	....	....	....	....
$H/+;\ ci\ ey^R/+ey^3$	128	1	3	....	....	191	....	....	....	....
$H/+;\ +/ey^3$	252	4	18	....	....	211	....	2	....	....
$ci\ ey^R/+ey^3$	178	....	....	....	....	188	....	....	....	....
$+/ey^3$	221	....	....	....	....	207	....	....	....	....
$+/+^1$	69	....	....	....	....	62	....	....	....	....
$H/+^1$	18	1	27	5	....	29	5	19	1	....
$ey^3/+^1$	89	....	....	....	....	92	....	....	....	....
$H/+;\ ey^3/+^1$	36	5	24	....	....	82	....	5	....	....
$+/+$	341	....	....	....	....	323	....	....	....	....
$H/+$	169	3	147	....	....	291	2	28	....	....
$+/ci$	377	....	....	....	....	384	....	....	....	....
$H/+;\ +/ci$	179	15	174	....	....	325	6	27	....	....

†The comparison, $H/+$ vs. $H/+;\ ey^3/+$ at 30°, shows a significant difference at the 1% level in both sexes. The comparison, $H/+$ vs. $H/+;\ +/ci$ at 26°, shows a significant difference at the 5% level in females, no significant difference in males.

[1] Genotypes developing at 30°.

$H/+;\ +/ci$. It would therefore appear that there is no enhancement or suppression of the action of $H/+$ on the fourth vein by $ci/+$ at 26°. This would imply that the suppression of the $H/+$ effect in $H/+;\ ci\ ey^R/+\ ey^2$ at 26° should be attributed to the presence of the ey^2 allele or of the compound ey^R/ey^2. If true this would require us to find out whether the presence of ey^R/ey^R in $H/+;\ ci\ ey^R/ci\ ey^R$ decreases the action of $H/+$ in this combination. That ey^R would have to affect H as a dominant modifier, if at all, has been indicated by the earlier comparison, $H/+;\ ci\ ey^R/ci\ ey^R$ vs. $H/+;\ ci\ ey^R/ci+$ (table 4). That this dominant modifier action, if it exists, is slight is suggested by the general similarity of the distributions of $H/+;\ ci/ci$ and $H/+;\ ci\ ey^R/ci\ ey^R$ of tables 1 and 3, respectively.

The effect of *en*/+ in combination with *H*/+ has been tested in a series of crosses, the results of which are given in table 9. A comparison of *en*/+; *H*/+ with *H*/+ of table 1 suggests a significant enhancement of the interruption effects of *H*/+ by *en*/+ for both males and females. The cross, *H*/+ × *en*/*en*,

TABLE 9

The interaction of Hairless with heterozygous engrailed.[1]

Genotype	Females					Males				
	0	1	2	3	4	0	1	2	3	4
en/+	564	....	....	....	....	514	....	....	....	....
en/+; *H*/+	6	....	412	31	....	76	11	407	....	....
Pm/+	200	....	....	....	....	206	....	....	....	....
en/+	237	....	....	....	....	215	....	....	....	....
Pm/+; *H*/+	145	3	48	....	....	157	3	17	....	....
en/+; *H*/+	24	1	187	7	....	53	3	128	1	....

[1] **The following comparisons show a significant difference at the 1% level in both sexes: *en*/+; *H*/+ vs. *H*/+ (table 1); *Pm*/+; *H*/+ vs. *en*/+; *H*/+.**

however, does not afford us a comparison between *en*/+; *H*/+ and *H*/+ where these two genotypes are subject to the same bottle environment and segregating genic backgrounds. In order to make this type of comparison the following cross was made: *Pm* +/+ *en*; *H*/+ × +/+. The results (table 9) show a significant increase in the degree of abnormality of L_4 venation in *en*/+; *H*/+ in comparison to *Pm*/+; *H*/+.

DISCUSSION

The results of the present study demonstrate that the effects on L_4 interruption of combinations of the *H*, *en* and *ci* mutants are greater than those expected by adding the effects of the single mutants comprising the combinations. These results could be interpreted in terms of super-additive interaction (NEEL 1941). There is, however, an alternative explanation.

If we take the percentage decrease from normality of *en*/*en*; *H*/+ and add to it the percentage decrease of *ci*/*ci*, we find a closer agreement with the decrease characteristic of the *en*/*en*; *H*/+; *ci*/*ci* genotype than when we add the decreases given by the *en*/*en*, *ci*/*ci* and *H*/+ genotypes taken singly. Likewise, the decrease for *en*/*en*; *ci*/*ci*, added to that of *H*/+, and the decrease of *H*/+; *ci*/*ci* added to that for *en*/*en*, approximate the percentage deficiency relative to normality due to the *en*/*en*; *H*/+; *ci*/*ci* genotype. These results show that an additive picture is more closely realized when an expected value for a three-mutant combination is arrived at by summing the action of one mutant and the action of the other two in combination, than when the individual actions of all three are summed. This is the situation to be expected if we are dealing with effects that are partly subthreshold in character, only part of the action of any one mutant being measurable on our scale of percentage interruption, there being a second invisible component of action due to the operation of a threshold involving some type of all-or-none reaction.

This type of interaction can be visualized if we assume that the three loci concerned are involved in some manner with the production of some material necessary for L_4 formation, and that the mutant substitution at each of the three loci causes a characteristic decrease in the amount of this substance. Furthermore, it would be necessary to assume that in the wild type fly the level of the postulated material lies some distance above that necessary for maximal L_4 venation (100%), and that when the level falls below this critical threshold value an interruption of the fourth longitudinal vein results.

When the actions of several mutants considered individually are used to predict their additive action in combination, an underestimate is arrived at due to the unmeasurable subthreshold components of each of the contributing mutants. However, if two mutants are combined and the action of one is sufficient to surpass the threshold, the entire action of the second then becomes measurable; the measurable action of the combination now being the sum of the measurable component of one mutant and the subthreshold and measurable components of the second mutant. With the addition of more mutants the relative error contributed by the one unmeasurable component necessary to reach the threshold level becomes less and we find a closer agreement between observed and expected values. Under this interpretation the enhancing action of the *en* and *ci* heterozygotes and hemizygous ci^+ would be explained on the basis of cumulative effects of the subthreshold decreases in vein material in these genotypes and the decrease of material characteristic of the modified homozygotes.

Since the interpretation of the data suggested above, involving cumulative actions ot subthreshold components of gene action, will be subjected to a detailed quantitative analysis in a subsequent paper, a more extensive discussion of the interactions of *en*, *ci* and *H* will be postponed until that time.

SUMMARY

The interaction of three mutants (cubitus interruptus, *ci;* engrailed, *en;* Hairless, *H*) producing an interruption of the fourth longitudinal vein (L_4) in *Drosophila melanogaster* has been investigated.

It is found that the three mutants interact superadditively in the various combinations, although an alternative explanation is possible involving additive action of postulated subthreshold components of mutant action.

Heterozygotes of *en* and *ci* act as enhancers of L_4 interruption in *ci* and *en* homozygotes respectively; also, hemizygous ci^+ enhances L_4 interruption in homozygous *en*. These heterozygous and hemizygous enhancements can be interpreted in terms of subthreshold differences between the homozygous wild type and +/en, +/ci, and $ci^+/-$.

The increase in normality of L_4 venation following the increase in dosage from one to two *ci* alleles is not affected by the substitution of the Hairless gene.

In some mutant combinations, venation effects not found in the single mutants appear.

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GENETIC AND ENVIRONMENTAL FACTORS IN THE TERATOGENIC EFFECTS OF BORIC ACID ON CHICKEN EMBRYOS[1]

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THERE is much variation in the extent to which hereditary and environmental agencies contribute to the origin of developmental malformations. At one end of the range we may postulate the existence of defects that arise in the presence of a single gene, the expression of which is independent of the residual genotype as well as of the environment. Such cases, if indeed on further study they are found to exist at all, are certainly rare, the great majority of mutants with teratological end-effects, whether they are dominant or recessive, being subject to genic modification. At the other extreme, and equally uncertain as a reality, one can envisage defects of purely environmental origin and for which variations in genetic resistance do not exist. In between these hypothetical limits, we find abnormalities produced by the interaction, in every conceivable combination, of environmental and hereditary forces.

We have shown recently (LANDAUER 1952) that an interesting syndrome of malformations can be produced by injecting, at the end of four days of development, boric acid into the yolk sac of chicken eggs. The resulting defects are, of course, environmental in nature par excellence. In such a situation it requires perhaps particular justification to study the participation of genetic factors in the manner and degree of response produced by the teratogenic substance. Because we believe that particular interest does, in fact, inhere in the nature of response differences to boric acid treatment, we have made observations on several breeds of fowl and on crosses between some of them. The results of these experiments are to be reported here.

MATERIALS AND METHODS

The stocks used for our experiments were White Leghorns (dominant white), White Minorcas (recessive white), Black Minorcas (solid black plumage) and Silver Gray Dorkings. As is commonly true, some of our White Minorca stock was heterozygous for dominant white. In addition to these "pure" stocks, we used eggs (F_1 embryos) from the following matings: reciprocal crosses between White and Black Minorcas, reciprocal crosses between White Minorcas and White Leghorns, and a cross of Black Minorca ♀♀ × White Leghorn ♂.

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All eggs were incubated in the same forced-draft incubator. After 96 hours of incubation, they received an injection into the yolk sac of 0.05 cc 5% boric acid (2.5 mg) dissolved in saline. Our records are based on all embryos and chicks surviving the 13th day of incubation. For the sake of greater conciseness, the descriptive records of malformations have been summarized into larger groups. Thus, the group of " minor beak defects " includes all cases of cross-beak, shortened maxilla, shortened mandible or parrot-beak; as " complex beak defects " we classified all cases in which a shortened mandible (sometimes in combination with twisting of the upper beak) was combined with cleft palate and/or facial coloboma (both at times unilateral); the heading of " tarsometatarsus defects " includes cases of shortening of the bone with or without bending; finally, under " toe defects " we have summarized all abnormalities of the toes ranging from a shortening of the fourth toe—the mildest expression of abnormality—to complete absence of all toes.

In order to provide a basis for evaluation of the incubation conditions and the incidence of sporadic malformations, we give in table 1 data for untreated control eggs. These came from the same matings and were incubated during the same period and in the same machine as were the experimental eggs. It can be seen that embryonic mortality was low and hatchability of the eggs excellent in all matings, except that of the Silver Gray Dorking fowl. In these latter, the higher mortality during incubation was presumably due to a certain measure of inbreeding during several preceding generations. The incidence of sporadic malformations was also highest among the Dorking embryos (2.3%); it was quite low in all other matings (0 to 1.2%).

TABLE 1

Embryonic mortality and incidence of malformations in untreated eggs of the matings used for experiments.

Mating	Number of fertile eggs	Mortality during incubation in %		Hatched	Malformations among survivors of 13th day
		To end of 2nd week	14–22 days		
				%	%
Black Minorca	376	5.6	9.1	85.4	0
White Minorca	220	3.2	4.5	92.3	0.5
Black Minorca ♀♀ × White Minorca ♂	335	5.4	6.3	88.4	0.6
White Minorca ♀♀ × Black Minorca ♂	249	2.4	7.6	90.0	0.8
White Minorca ♀♀ × White Leghorn ♂	266	4.1	6.0	89.8	1.2
White Leghorn ♀♀ × White Minorca ♂	353	2.5	5.1	92.4	0.3
Black Minorca ♀♀ × White Leghorn ♂	305	3.9	7.6	88.5	0.3
Silver Gray Dorking	307	14.0	19.2	66.8	2.3

The effects of boric-acid treatment at 96 hours of incubation on embryos from these matings are shown in table 2. These data are the results for the whole experimental period, viz. for eggs put into the incubator from February 12 to May 20, 1952.

Seasonal trends

In order to determine if conditions changing with the season had any influence on the response of embryos to boric acid, the data from each of the different breeds and crosses were divided into two groups for comparison, those from settings between February 12 and April 1 representing the early part of the season, those from settings between April 15 and May 20 forming the results for the later part of spring.

Analysis of these data revealed some very striking seasonal differences in response to boric acid. This was particularly true for the data from Black Minorcas. The incidence of complex beak defects in Black Minorca embryos was 57.6 percent during the early part of the season, but only 32.1 percent in

TABLE 2

Embryo mortality and incidence of malformations after injection of 2.5 mg boric acid at 96 hours of incubation. Summary of results for whole season.

Mortality and hatching in percent of number treated; curled toe paralysis in percent of hatched chicks; malformations in percent of survivors of 13th day.

	B.M.	W.M.	B.M.♀♀ × W.M.♂	W.M.♀♀ × B.M.♂	W.M.♀♀ × W.L.♂	W.L.♀♀ × W.M.♂	B.M.♀♀ × W.L.♂	S.G.D.	W.L.
Number treated	656	414	569	494	533	667	556	372	345
Mortality from 96 hours to end of 2nd week %	22.3	15.0	19.3	23.9	19.1	21.3	16.9	36.6	38.0
Mortality 14–22 days %	62.2	65.5	65.2	53.4	61.9	58.3	56.3	62.6	49.3
Hatched %	15.5	19.6	15.5	22.7	18.9	20.4	26.8	0.8	12.8
Curled toe paralysis (among hatched) %	43.2	44.4	45.5	34.8	35.6	50.7	43.0	....	36.4
Number surviving 13th day	510	352	459	376	431	525	462	238	214
Simple beak defects %	22.9	14.8	28.5	33.5	26.5	11.6	24.5	2.5	10.3
Complex beak defects %	43.9	53.7	40.3	18.4	46.9	54.3	38.7	91.2	50.0
Tibiotarsus bent %	4.3	1.1	2.6	1.3	4.2	1.0	1.5	0.8	3.7
Tarsometatarsus defects %	29.6	23.9	15.3	16.8	19.0	19.2	13.4	50.8	23.4
Toe defects %	14.7	11.4	10.9	6.9	10.0	9.9	14.5	22.7	20.6
Normal %	24.7	21.6	26.6	35.4	21.3	22.9	28.6	4.2	28.5

Breed designations: B.M.= Black Minorca, W.M. = White Minorca, W.L. = White Leghorn, S.G.D. = Silver Gray Dorking.

late spring ($\chi^2 = 33.41$; $P < .0001$). The reverse was true for the single beak defects, the incidence having been 13.6 percent early, but 31.0 percent late (difference = 17.4 ± 3.57%). As with the complex beak defects, malformations of the tarsometatarsus declined among the Black Minorca embryos with advancement of the season; during early spring the incidence was 45.3 percent, but only 16.1 percent during late spring ($\chi^2 = 52.08$; $P < .0001$).

The experiments with White Minorca embryos yielded a similar, though less pronounced, seasonal dependence of the complex beak abnormalities. They declined from 63.4 to 47.7 percent ($\chi^2 = 8.32$; $P < .01$). The simple beak defects rose in incidence, but the difference was not significant. There was no significant seasonal change in incidence of tarsometatarsus defects.

No clear seasonal trends were discernible among White Leghorn embryos for any of the boric-acid-induced malformations. The various crosses between breeds and the Silver Gray Dorkings all showed a seasonal decline of complex beak defects and a rise in the simple beak abnormalities, but none of the differences were significant.

Intra-breed heterogeneity

For a given breed or cross and within a limited span of time the incidence of particular malformations, produced by boric-acid treatment, remains relatively constant. We were interested to determine if this comparative constancy of response was brought about by uniformity of behavior of the progenies from different mothers or if it existed in spite of heterogeneity between them. The question was tested on the results with embryos of Black and White Minorca fowl. The analysis was based on eggs put into the incubator between February 12 and March 18, and was directed toward the question whether heterogeneity

TABLE 3

Individual records of Black Minorca females in regard to response of their progenies to the injection of 2.5 mg boric acid at 96 hours of incubation.

Mother's number	Total progeny	Free of malformations	Beak defects of any kind	Tarsometatarsus defects
1498	9	2	5	3
1500	7	3	4	2
01	6	1	5	3
02	10	2	8	5
03	7	1	6	4
04	10	3	7	4
05	10	7	3	1
06	6	1	5	4
07	11	0	11	11
08	14	3	11	5
09	7	2	5	4
10	11	1	10	9
11	11	6	5	4
12	5	0	5	4
13	10	1	9	8
14	13	4	8	6
15	12	2	10	9
16	4	0	3	4

between mothers existed in the following three features: (1) freedom from all malformations; (2) abnormalities of the beak (any kind); (3) defects of the tarsometatarsus. The relevant data for Black Minorcas are shown in table 3.

Tests for heterogeneity of these data gave, with 17 degrees of freedom, the following results: freedom from malformations $\chi^2 = 29.21$; P about 0.025; occurrence of beak defects $\chi^2 = 28.71$, P about 0.025; presence of malformations of the tarsometatarsus $\chi^2 = 38.59$, $P < 0.01$. Since some of the progenies were very small, tests for heterogeneity were repeated by limiting the analysis to the ten hens that had had progenies of at least ten embryos. The results thus obtained show an even higher level of significance (all $P < .01$). It is clear, therefore, that there were significant differences between Black Minorca mothers in regard to the response of their progenies to boric-acid treatment.

A similar analysis of our data for White Minorca progenies, but with the size of the total sample considerably smaller than that from Black Minorcas, brought evidence for highly significant heterogeneity in the production of tarsometatarsus malformations ($\chi^2 = 32.92$; Df = 12; $P < 0.01$). The White Minorca progenies showed no significant heterogeneity, however, as far as beak defects or freedom from all malformations were concerned.

Breed response differences

It is the most outstanding feature of our data that the Silver Gray Dorking embryos responded to boric-acid treatment with a much higher incidence of malformations than any of the other breeds and stocks tested. A comparison with the data for White Leghorns showed that complex beak defects and malformations of the tarsometatarsus were produced with much higher frequency in the Silver Gray Dorking stock, and this was true during the early as well as the late part of the experimental season. Abnormalities of the toes were in the Dorking stock significantly more common during early spring, but during the later part of the season the differences were not significant, though the trend was in the same direction.

The Silver Gray Dorkings differed from the Black Minorcas in a manner very similar to that just described in comparison to the data for White Leghorns, except that during the early part of the season the differences between the two stocks were not significant in incidence of malformations of tarsometatarsus and toes, but became highly significant during the second half of the season. The data for Silver Gray Dorking and Black Minorca embryos are given in table 4. A comparison of the results for Dorking embryos with those obtained from White Minorca stock and from the various crosses yielded differences of a similar nature, and these need not be discussed in detail.

It might be asked if the greater response of the Dorking embryos to boric acid was the consequence of a heightened " debility " of the stock on account of inbreeding. There had, however, been only a minor degree of inbreeding and, what is even more to the point, Dorking embryos did by no means give uniformly greater response to all teratogenic substances. The effect of insulin on the extremities, for instance, did not differ between Dorking and White

TABLE 4

Differences, in response to boric acid treatment, between Black Minorca and Silver Gray Dorking embryos. The size of the samples on which the percentages are based is shown in parentheses.

Malformations	Black Minorca %	Silver Gray Dorking %	Significance	
			χ^2	P
Complex beak defects				
Early	57.6 (236)	92.2 (141)	50.74	<.0001
Late	32.1 (274)	89.7 (97)	78.10	<.0001
Whole season	43.9 (510)	91.2 (238)	149.82	<.0001
Tarsometatarsus defects				
Early	45.3 (236)	53.2 (141)	2.16	.14
Late	16.1 (274)	47.4 (97)	38.48	.0001
Whole season	29.6 (510)	50.8 (238)	31.70	.0001
Toe defects				
Early	17.4 (236)	18.4 (141)	0.078	about .80
Late	12.4 (274)	28.9 (97)	13.97	<.001
Whole season	14.7 (510)	22.7 (238)	7.30	<.01

Leghorn (table 7) or Minorca embryos. The most reasonable explanation of the dissimilarities in response to boric acid would, therefore, seem to be that we are dealing with real stock or breed differences.

Boric-acid treatment did not lead to significant differences between Black Minorca and White Leghorn embryos in the incidence of (complex or single) beak defects. This was true in early and late spring. The same applies to Black versus White Minorcas, except that during late spring the White Minorca embryos showed a significantly higher incidence of complex beak defects ($\chi^2 = 12.01$; $P < .001$). This situation is explained by the fact that a sharp seasonal decline of complex beak defects (and an increase in simple beak abnormalities) occurred in Black Minorcas, but that only relatively slight changes of the same kind took place in White Minorcas.

The frequency of tarsometatarsus abnormalities in response to boric-acid treatment was significantly higher in Black Minorcas than in White Leghorns during the early part of spring ($\chi^2 = 57.70$; $P < .0001$) and for the whole season ($\chi^2 = 32.39$; $P < .0001$), but (because of the seasonal decline in incidence among Black Minorcas) these differences disappeared during late spring. A very similar situation is revealed by a comparison of the data for Black and White Minorcas, namely, a great excess among Black Minorcas of tarsometatarsus defects during early spring ($\chi^2 = 19.28$; $P < .0001$), with a reversal of the situation in late spring (because of a seasonal decline in response among the Black Minorcas, but not the White Minorcas), resulting in an actually higher incidence among the White Minorca embryos at this time ($\chi^2 = 5.80$; $P < .02$). The data for toe defects in Black and White Minorca embryos showed trends of incidence similar to those for tarsometatarsus abnormalities, but the differences were not statistically significant.

During the early part of the season boric-acid treatment produced complex beak defects with much higher frequency in White Minorca than in White

Leghorn embryos ($\chi^2 = 17.73$; $P < .0001$), but the two breeds did not show significant response differences in any other respect.

The effect of outcrossing Black and White Minorcas to White Leghorns on response to boric-acid treatment

In comparing the responses to boric acid of Black and White Minorca embryos, respectively, with those of White Leghorn embryos, some interesting differences have been reported above. The principal dissimilarities between the two Minorca breeds on the one hand and White Leghorns on the other, were found in the fact that during early spring Black Minorca embryos gave a much higher incidence of tarsometatarsus abnormalities than did White Leghorn

TABLE 5

The incidence of complex beak and of tarsometatarsus defects, produced by boric acid during early and late spring, in Black and White Minorca embryos and in F_1 embryos of Black Minorca ♀♀ × White Leghorn ♂ and White Minorca ♀♀ × White Leghorn ♂. The size of the samples on which the percentages of beak and tarsometatarsus defects are based is shown in parentheses.

Mating	Complex beak defects Incidence in %		Significance of differences in *early* spring	Tarsometatarsus defects Incidence in %		Significance of differences in *early* spring
	Early spring	Late spring		Early spring	Late spring	
B.M.	57.6 (236)	32.1 (274)	$\chi^2 = 9.88$ $P < .002$	45.3	16.1	$\chi^2 = 35.40$ P .0001
B.M.♀♀ × W.L.♂	42.3 (189)	36.3 (273)		18.0	10.3	
W.M.	63.4 (134)	47.7 (218)	$\chi^2 = .61$ P abt. .42	22.4	24.8	$\chi^2 = .001$ P abt. 1.0
W.M.♀♀ × W.L.♂	58.9 (151)	40.4 (280)		22.5	17.1	

Breed designations: B.M. = Black Minorca, W.M. = White Minorca, W.L. = White Leghorn.

embryos, whereas White Minorca embryos responded with a higher incidence of complex beak defects than did White Leghorn embryos. In other respects, embryos of the two Minorca breeds did not significantly differ from White Leghorns.

Some of the results obtained with F_1 embryos of Black Minorca ♀♀ × White Leghorn ♂ and of White Minorca ♀♀ × White Leghorn ♂, as compared with the maternal Minorca breeds, are shown in table 5. During the early part of spring, outcrossing of Black Minorca females to a White Leghorn male led to a great reduction in incidence of complex beak and of tarsometatarsus defects. Outcrossing White Minorca females to a White Leghorn male, on the other hand, if compared with the White Minorca data, did not result in significant changes of response to boric acid. During late spring neither of the two outcrosses differed significantly from the maternal breeds in incidence of complex

beak defects, and both showed a slight, but probably significant lowering in the frequency of tarsometatarsus abnormalities (the χ^2 being 4.66 and 4.33, respectively, with a P in either case of about .03).

Plumage color and response to boric acid

According to preliminary reports by BERNIER (1948–1950), chickens with black plumage have higher riboflavin requirements than white-feathered ones. Since our earlier observations (LANDAUER 1952) had shown that malformations induced by boric acid are related to disturbances of riboflavin utilization, it seemed of interest to determine if differences in response to boric acid, comparable to those found by BERNIER, exist between embryos which are dissimilar in their genotype for down color or between embryos from (or eggs laid by) mothers with dissimilar plumage color.

The fact that the most extreme responses to boric acid were shown by embryos from the two colored breeds used in our tests, namely, Silver Gray

TABLE 6

Data comparing the response to boric acid of phenotypically white embryos segregating in reciprocal crosses of Black and White Minorca fowl. Incidence of curled toe paralysis in percent of hatched chicks; incidence of other traits in percent of survivors of 13th day of incubation. The size of the samples on which the percentages are based is shown in parentheses.

	White embryos and chicks		Significance of difference	
	19 white mothers	20 black mothers	χ^2	P
Curled toe paralysis (among hatched) %	6.7 (90)	48.5 (33)	28.77	<.0001
Free of malformations %	40.7 (268)	28.9 (204)	6.97	<.01
Complex beak defects %	17.2 (268)	38.2 (204)	26.54	<.0001
Toe defects %	3.4 (268)	11.5 (204)	9.28	<.01

Dorkings and Black Minorcas, is suggestive rather than compelling evidence; for, we might deal with breed differences that are determined by genetic factors unrelated to pigmentation. It was, therefore, necessary to search for other evidence with a bearing on our problem. Such evidence was provided by our data from reciprocal crosses between Black and White Minorca fowl in which, as stated earlier, segregation of embryos with black and with white down occurred. In order to minimize the chances of the results being influenced by the sire, we used in each of the two pens three different cocks in rotation during the first and again during the second part of the hatching season. There was no suggestion in the data that the results had been influenced by the use of different sires.

Table 6 gives data for all phenotypically white embryos and chicks produced by mothers with either white or black plumage. It can be seen that considerably fewer white chicks from black than from white mothers were entirely free of malformations. Furthermore, it is evident that in white chicks from black mothers, as compared with those from white mothers, boric-acid treatment was

responsible for a much higher incidence of curled toe paralysis, complex beak defects and malformations of the toes. All these differences are of high statistical significance. Similarly, it was found that among black embryos and chicks from white mothers the incidence of complex beak defects was 18.8 percent, whereas for black chicks from black mothers the corresponding figure was 42.3 percent (χ^2 for difference = 16.81, with $P < .0001$).

Another comparison may be made by contrasting the responses of the embryos according to their phenotypical down coloration, but without reference to their mothers' plumage color. In thus combining the data for the two reciprocal matings between Black and White Minorca fowl, it is found that more white than black embryos and chicks were free of malformations ($\chi^2 = 12.07$; $P < .001$) and that major beak defects were probably, during the early part of spring, significantly more common among black embryos and chicks than among the white ones ($\chi^2 = 4.34$; $P = .035$). There were no significant differences between the two groups in the incidence of abnormalities of tarsometatarsus and toes. On the whole, it is clear that the mother's plumage color is a much more potent factor in determining the embryo's susceptibility and teratogenic response to boric acid than is its own prospective down color. No significant response differences occurred in reciprocal crosses which did not involve dissimilarity of plumage color (White Minorca × White Leghorn).

DISCUSSION

It has been reported on earlier occasions that in the experimental production of malformations the outcome is influenced by genetic factors as well as by conditions of the environment. Intra-breed differences in teratogenic response to mechanical shaking and to the injection of insulin are known to exist among White Leghorn hens (LANDAUER and BAUMANN 1943; LANDAUER and BLISS 1943). Dissimilarities in teratogenic reaction to selenium-containing feed and to injection of insulin were observed between different breeds and stocks (LANDAUER 1940, 1948). In the case of insulin-induced rumplessness reciprocal crosses between breeds with greatly differing responses have shown that maternal influences play an important role in determining susceptibility of the progeny (LANDAUER 1948). The incidence of beak defects and micromelia after insulin treatment was found to decline from early to late spring (LANDAUER and RHODES 1952). KALTER and FRASER (1952) have similarly reported recently that in mice the *in utero* production of cleft palate by treatment of the pregnant mothers with cortisone is influenced by genetic and maternal factors, the incidence of the malformation differing between strains and a genetically influenced maternal-fetal relationship contributing in some manner to the production of cleft palate by cortisone.

These findings should not really be an occasion for great surprise. Since the maintenance of life against the manifold adversities of the internal and external environment is the mainspring of evolution, the existence of a great variety of protective mechanisms should be expected, and the presence of some of these will only be revealed under particular conditions of stress. The really intriguing

problems, about which we know very little, relate to the physiological processes that bring about varying degrees of teratological response and to the manner in which these processes act either upon the organism as a whole or on special parts. It is in this field that our present observations seem to contribute some information.

In considering the implications of our results, the following facts should be taken into account: (1) Earlier observations render it likely that boric acid produces its teratological effects by an interference with riboflavin-containing enzymes (LANDAUER 1952). (2) Within and between breeds differences in riboflavin requirements or in response to riboflavin supplements are known to exist (DAVIS, NORRIS and HEUSER 1938; LAMOREUX and HUTT 1948; LERNER and BIRD 1948). (3) The occurrence of seasonal trends in the chemical composition of eggs has been demonstrated (CSONKA 1950). (4) Black chickens apparently have higher riboflavin requirements than white ones (BERNIER 1948–50). (5) An increase in the riboflavin concentration of eggs has been observed during late winter and early spring (JACKSON *et al.* 1946).

Our present observations suggest not only that the teratogenic effectiveness of boric acid differs within and between breeds, but also that some of these variations are accounted for by physiological processes associated with plumage pigmentation. Pigment formation is not yet under way in the embryos during the developmental stages in which boric acid has teratogenic effects. (If, however, there is competition for an essential substrate or coenzyme between the affected organs and the melanoblasts, evidence of it should during later stages be found in down pigmentation of colored embryos. That this is, indeed, the case, will be shown in another publication, LANDAUER 1953.) The clearest indication for a relationship between plumage pigmentation and the effects of boric acid came from reciprocal crosses between Black and White Minorca fowl. Since the white parents were heterozygous for recessive and dominant white, segregation for black and white down occurred among the progeny of these crosses, and it could be shown that the prospectively white (and also the black) embryos from *black* mothers were more susceptible to the teratogenic action of boric acid than those from white mothers.

This "maternal" effect of plumage color on susceptibility of the embryos is presumably related to chemical composition of the eggs. Taking into consideration the facts reviewed previously, it seems plausible to suspect that differences in riboflavin content of the eggs laid by colored and white hens influence the extent to which embryos are able to withstand the action of boric acid. It is also noteworthy that the seasonal trend (from early to late spring) toward a lower incidence of malformations (after boric-acid treatment) was most evident in the progenies of mothers with pigmented plumage, especially the Black Minorcas. No similar trend was discernible in White Leghorn stock, and in White Minorcas the changes were much less marked than in Black Minorcas. It may be assumed that, as in other respects, with advancing spring (higher ambient temperatures) the maternal organism of chickens can better afford to spare critical compounds (in the present instance presumably ribo-

flavin) for incorporation into their eggs, and it is not surprising that hens with pigmented plumage are more influenced by these environmental changes than are white hens.

The difference between colored and white breeds in the seasonal decline of malformations after boric-acid treatment is especially marked in the case of the tarsometatarsus. In this instance, the trend exists only among the progenies of colored fowl. Additional evidence exists in our data for the conclusion that the teratological responses of the tarsometatarsus (and probably of the toes as well) have features that differentiate them from those of the facial skeleton.

TABLE 7

Comparative data for the teratological responses of White Leghorn and Silver Gray Dorking embryos to treatment with boric acid and insulin. The facial defects include all abnormalities of beak and palate; malformations of the extremities are represented by tarsometatarsus defects after boric acid, by micromelia after insulin.

Treatment	White Leghorn			Silver Gray Dorking		
	N	Facial defects %	Abnormal extremities %	N	Facial defects %	Abnormal extremities %
Boric acid 2.5 mg 96 hrs.	214	60.3 ± 3.42	23.4 ± 2.97	238	93.7 ± 1.57	50.8 ± 3.23
Insulin 2 U 96 hrs.	118	16.1 ± 3.35	29.7 ± 4.18	65	29.2 ± 5.64	18.5 ± 4.82
Insulin 5 U 96 hrs.	79	43.0 ± 5.54	79.7 ± 4.47	60	58.3 ± 6.37	71.7 ± 5.80
Insulin 2 U 120 hrs.	180	13.9 ± 2.58	46.7 ± 3.72	58	36.2 ± 6.20	51.7 ± 6.45
Insulin 5 U 120 hrs.	76	50.0 ± 5.72	93.4 ± 2.85	45	64.4 ± 7.17	93.3 ± 3.74

Intra-breed heterogeneity between treated progenies of different mothers, for instance, is more marked for defects of the tarsometatarsus than for abnormalities of the beak. It is not clear at present whether these dissimilarities of response are merely an expression of quantitative differences in susceptibility of the respective tissues or whether they are consequences of qualitative diversity in the physiological functions of the affected parts.

It is known, however, from earlier studies (LANDAUER and RHODES 1952) that local specificities of reaction to teratogenic substances do, in fact, exist. Additional evidence for the same conclusion may here find its place. Table 7 contrasts the reactions to boric acid and insulin of White Leghorn and Silver Gray Dorking embryos, as expressed by incidence of facial defects and of abnormalities of the extremities. Several facts emerge clearly from a scrutiny of

these data and their comparison with facts already set forth in the present report: (1) that, as was pointed out at an earlier occasion, the responses to boric acid and insulin differed quantitatively (as well as qualitatively) between the two regions, the facial parts showing a higher incidence of malformations than the extremities after boric acid treatment, but the reverse holding after insulin injection; (2) that Silver Gray Dorking embryos reacted in both body regions to boric acid with a much higher incidence of abnormalities than did White Leghorn embryos; (3) that, in contrast to boric acid, the incidence of abnormal extremities brought about by insulin treatment did not differ significantly between the two breeds, whereas the frequency of facial defects produced by insulin was, as in the case of boric acid, consistently higher in Dorking as compared with Leghorn embryos. It follows from these observations that various parts of embryos may give dissimilar quantitative responses to one and the same or to different teratogenic substances, and it is these dissimilarities, as well as the total incidence of all types of defects, that may become exaggerated in the presence of maternal plumage pigmentation. There is little doubt that we are dealing with competition phenomena and it seems likely that agencies associated with maternal (and to a lesser degree embryonic) pigmentation serve to exacerbate the situation.

SUMMARY

A comparative study was made concerning the teratogenic effects of boric acid, injected into the yolk sac of 96-hour embryos belonging to several different breeds of fowl and crosses between them. Eggs of the following stocks were used: Silver Gray Dorking, White Leghorn, Black and White Minorca, reciprocal crosses between the two Minorca stocks and between White Leghorn and White Minorca, as well as from Black Minorca ♀♀ × White Leghorn ♂. The majority of White Minorcas were heterozygous for dominant white plumage. The principal results were as follows:

1. A distinct seasonal trend in teratological response to boric acid was found among the embryos of some stocks, the incidence of induced malformations declining from early to late spring. This trend was most marked in the Black Minorca data.

2. Significant heterogeneity in response to boric acid was shown to exist between progenies of different mothers belonging to one and the same breed.

3. Highly significant differences in response to boric-acid treatment were found between breeds. The two colored breeds used in our tests, namely, Silver Gray Dorkings and Black Minorcas, gave a higher incidence of malformations than did the white breeds.

4. Outcrossing of Black Minorca females to a White Leghorn male resulted in a great lowering in incidence of complex beak and of tarsometatarsus defects among the F_1 embryos. The same did not hold for outcrosses of White Minorca females to a White Leghorn male.

5. Plumage color of the mother had a definite effect on response of her progeny to boric acid. In segregating progenies it was found that white em-

bryos from black mothers showed more malformations than white embryos from white mothers. The phenotypical down color of the embryos themselves has an effect of the same nature, but to a much lesser degree.

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EVIDENCE FOR IRREGULARITY IN CROSSING OVER OF THE S LOCUS IN THE EVERSPORTING TYPE OF MATTHIOLA INCANA (L.) R. BR.

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THE double-flowered type in stocks (*Matthiola incana*) is completely sterile, hence can be obtained only from heterozygous singles. Two genetic types of heterozygous singles are readily recognized from their breeding behavior: (1) normal heterozygous singles which on selfing produce 3 singles : 1 double and (2) eversporting singles which on selfing continue through successive generations to produce about 1 single : 1 double with a slight but consistent excess of doubles.

In reciprocal crosses between eversporting and true-breeding singles SAUNDERS (1911, 1928) found that in both crosses the F_1 plants were single-flowered. When a true-breeding single was used as the female, all of the F_1 plants produced about 3 singles : 1 double in the F_2 generation, but when an eversporting single was used as the female only half of the F_1 plants gave ratios of about 3 : 1 in the F_2 while the other half gave all singles. This evidence demonstrates that eversporting singles are maintained heterozygous by the failure of the singleness gene to be transmitted through the pollen, whereas both singleness and doubleness genes are transmitted through the eggs. That phenomenon accounts for the recurrent 1 : 1 ratio obtained on selfing eversporting singles.

The nonfunction of pollen carrying the singleness factor in the eversporting type was further substantiated by the work of SNOW (1925) and WADDINGTON (1929) who found that the percentage of pollen germination on artificial media was much less for the eversporting type than for the normal heterozygous type. This was more conclusively demonstrated by SNOW (1924, 1925) and KUHN (1937) who by counted-grain pollination showed that only about half as many pollen grains from eversporting stocks as compared with normal heterozygous stocks were functional in inducing seed set.

To explain the consistent excess of doubles in the eversporting type SAUNDERS (1911) assumed that two genes X and Y are both necessary for singleness. In the normal heterozygous type ($Xx Yy$) these genes are completely linked while in the eversporting type (also $Xx Yy$) they recombine to produce a gametic ratio of 15 XY : 1 Xy : 1 xY : 15 xy. All of these gametic genotypes were assumed to be transmissible through the egg but only the xy combination through the pollen. Under these conditions the ratio of 15 singles : 17 doubles expected in the progeny would account for an excess of doubles over singles in eversporting lines.

Objections to this theory which more recently have been adequately discussed by WINGE (1931) and KAPPERT (1937) led to the proposal of the pollen lethal hypothesis by GOLDSCHMIDT (1913) and FROST (1915). According to FROST's interpretation of that hypothesis single versus double flower form in the normal heterozygous type of *Matthiola incana* is governed by one gene pair *Ss*, whereas in the eversporting type the singleness gene *S* either has mutated to the lethal allele S^l or a lethal factor at another locus is so closely linked with *S* that crossing over is not known to take place between them. The lethal factor was assumed to render nonfunctional all of the pollen grains carrying it and to reduce slightly the viability of the eggs or zygotes carrying it. Thus the verifiable nonfunction of one class of pollen as well as the observed excess of doubles were attributed to the effect of one factor, as indicated by the following diagram:

Eversporting single

Sl/s+ **(selfed)** ⟶

	Pollen		
Eggs	*(Sl)*	*(s+)*	
(Sl)	—	*Sl/s+*	**Singles**
(s+)	—	*s+/s+*	**Doubles (excess)**

Furthermore, by this hypothesis the commonly observed lower vigor of the singles than of the doubles in eversporting lines (SAUNDERS 1911; FROST 1915) could be explained logically as a consequence of the singles only carrying the lethal factor.

WINGE (1931) presented evidence which was interpreted to show that linkage between *S* and *l* is not complete. This evidence consisted of recovering a plant of the genotype *S+/s+* in the progeny of a selfed eversporting single. As shown in table 1, crossing over between *S* and *l* theoretically could yield five new genotypes in such a progeny among which *S+/s+* would be expected to occur with a greater frequency than any of the others since it requires but one crossover gamete contributed by either egg or pollen. This genotype which represents the normal heterozygous single was previously known, and is frequently found in populations of *Matthiola incana* that are not rigidly selected

TABLE 1

Noncrossover and postulated crossover genotypes obtainable from selfing an eversporting (Sl/s+) plant of Matthiola incana.

Eggs		Pollen			
		Noncrossovers		Crossovers	
		(Sl)	*(s+)*	*(S+)*	*(sl)*
Noncrossovers	*(Sl)*	—	*Sl/s+*	*Sl/S+*	—
	(s+)	—	*s+/s+*	*S+/s+*	—
Crossovers	*(S+)*	—	*S+/s+*	*S+/S+*	—
	(sl)	—	*sl/s+*	*S+/sl*	—

for the eversporting habit. However, its occurrence in the progeny of an eversporting plant afforded provisional evidence of crossing over between *S* and *l*.

Recovery of other postulated crossover genotypes in the immediate progeny of an eversporting plant would provide added provisional evidence that *S* and *l* involve separate loci. The probability of recovering the genotypes *S+/S+* and *S+/sl*, however, would be exceedingly small since each would require the combination of two crossover gametes. The genotype *sl/s+* would be impossible to verify by direct genetic methods since it would represent a double-flowered, sterile plant. The remaining crossover genotype *Sl/S+* would breed true for singleness. Its complete verification would depend upon demonstrating that it does in fact contain *l*.

KAPPERT (1937) presented indirect evidence of the recovery of that genotype in eversporting lines by following the apparent effect of *l* on a morphological character rather than its effect on the ratio of singles to doubles. In one of his lines, for example, the singles consisted of 1334 short-capsuled and 34 long-capsuled plants. In progeny tests of a number of plants of each phenotype the long-capsuled plants gave 3 singles : 1 double, indicating a genotype of *S+/s+* with respect to the factors affecting the frequencies of singles and doubles. The short-capsuled plants gave mostly a ratio of 1 : 1, indicating a genotype of *Sl/s+*. From this it was concluded that the lethal factor affected capsule length. Hence, when 2 short-capsuled plants out of 133 tested produced all singles instead of the characteristic 1 : 1 ratio, it was further concluded that these plants were of the genotype *Sl/S+*. Progenies from presumed *Sl/S+* plants showed a deficiency of short-capsuled individuals which corresponded with the observed deficiency of doubles in progenies of *Sl/s+* plants.

The validity of this evidence obviously rests upon the premise that the observed difference in capsule length is governed by *l* rather than by another factor closely linked with *l*. KAPPERT (1951) later pointed out that lines vary in the reduction of capsule length attributed to *l* and that the effect of the lethal factor is perceivable only if the lines are sufficiently homozygous with respect to specific length factors.

The experiment described in the present study provides direct genetic evidence for the recovery of the genotype provisionally designated *Sl/S+* in the immediate progeny of an eversporting plant. The provisional crossover chromosome *S+* and the noncrossover chromosome *Sl* were tested for the presence of *l* by combining each with a homolog carrying *s+*. The presence of *l* could then be detected by its effect on the ratio of singles to doubles in subsequent progeny tests. This method thus utilized the basic criterion for *l* in its identification.

EXPERIMENTAL RESULTS AND DISCUSSION

Recovery of postulated crossover types

Among five progenies from individual plants selfed in eversporting line B2479 one progeny (5388) contained all singles and the rest showed the characteristic eversporting ratio (table 2). By reference to table 1 it may be seen

TABLE 2

Number of single- and double-flowered plants in eversporting line B2479 and in 5 progenies from individual plants selfed in that line.

Culture No.	Number of plants		Percentage of doubles
	Singles	Doubles	
B2479	17	24	58.5
5388	207	0	0
5389	73	139	65.6
5392	144	237	62.2
5393	60	85	58.6
5394	44	71	61.7

that a true-breeding single occurring in the immediate progeny of an eversporting plant might represent one of three possible genotypes, *Sl/S+*, *S+/S+* and *S+/sl*. The first of these presumes a crossover in the microsporocyte only, and its probability of occurrence is much greater than that of either of the last two, each of which requires the combination of two crossover gametes. Hence there is a great likelihood that the parent plant of progeny 5388 represented the genotype *Sl/S+* rather than *S+/S+* or *S+/sl*.

In order to test progeny 5388 for the genotype of its parent 24 plants in that progeny were crossed with eversporting (*Sl/s+*) plants used as males. To facilitate distinction between authentic F_1 hybrids and possible selfs these males were selected so as to produce an F_1 flower color intermediate between those of the parents. The purpose of this cross was to reintroduce the doubleness gene in order that the presence of the lethal factor might be detected in the F_1 or F_2 by its effect on the ratio of doubles to singles. The number of plants obtained in the F_1 from each of these crosses is given in table 3. The percentage of doubles in each of 273 F_2 progenies obtained from selfing a number of plants in each of the F_1 progenies is also given in that table. In table 4 the F_2 progenies are classified as to frequency and observed percentage of doubles in two expected genetic classes.

The genetic expectation in the F_1 and F_2 generations following the cross described is given in table 5 for each of the three possible true-breeding single genotypes obtainable as a result of crossing over between *S* and *l* in a selfed eversporting plant. On the assumption that the parent plant of progeny 5388 was of the genotype *S+/sl*, somewhat more than half of the plants in progeny 5388 would be expected to be of the genotype *S+/S+* and to produce pure single progenies (*S+/s+*) in the F_1 when crossed with an eversporting male. Somewhat less than half would be expected to be of the genotype *S+/sl* and to produce progenies exhibiting approximately 1 single (*S+/s+*) : 1 double (*sl/s+*) in the F_1. The data in table 3 show that this was not the case for the plants in progeny 5388 which actually yielded essentially pure single progenies in the F_1 for all but one of the 23 plants tested. Therefore, *S+/sl* is eliminated as a possible genotype for the parent of that progeny.

The one exceptional plant (5388-20) gave an unexpected ratio of 19 singles : 18 doubles in the F_1. The flower color of the entire F_1 progeny showed that

TABLE 3

Frequency of double- and single-flowered plants in the F_1 and F_2 progenies from 23 plants in pure-single progeny 5388 crossed with eversporting males.

Identification no. of plants in pure-single progeny 5388	Number of plants obtained in the F_1 progenies		Frequency of reared F_2 progenies showing indicated percentages of doubles (Class centers in percent)														Total F_2 plants
	Singles	Doubles	7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5	62.5	67.5	72.5	77.5	
5388-1	2					2												362
" -2	10				2	3	3	1										1293
" -3	55	1				5	5	1			1	5	2	1				2283
" -4	12					5	3					3		1				1715
" -5	10	1					4	1			1	1	1	1				1415
" -6	46					5	4		1									1255
" -7	47				2	5	4	1										1537
" -8	50		2			6	5	1										1671
" -9	11				1	6	3											1486
" -10	54				3	8	5	2										2235
" -11	12					4	4	2					1	1				1553
" -12	12	2			1	2	3					2	1	1				912
" -13	45				2	7	3											1155
" -14	15				1	4	5	5										1428
" -15	17					4	6	2		1	1		2	1				2344
" -16	11				1	7	3											1447
" -17	12					5	1				1	2	1	1			1	1081
" -18	44			2		2	6	1										1557
" -19	48				2	4	5	1										1254
" -20	19	18			1		3											542
" -22	51				1	7	5	3										1765
" -23	40	2			1	3	2			1	1		2					914
" -24	4				3	6	4	2										1692
	627	25	2	2	21	100	86	23	1	2	5	13	10	7			1	32896

TABLE 4

Classified F_2 data from 23 plants in pure-single progeny 5388 crossed with eversporting males.

Identification no. of plants in pure-single progeny 5388	F_2 progenies classified according to expected genetic ratios					
	No. of progenies in each class		Observed mean percentage doubles in each class		P from χ^2 for homogeneity among progenies in each class	
	3:1	1:1	3:1	1:1	3:1	1:1
5388-1	2		21.8 ± 2.3		.30–.50	
" -2	9		24.0 ± 1.2		.05–.10	
" -3	11	9	24.6 ± 1.2	53.0 ± 1.6	.80–.90	.50–.70
" -4	8	4	24.1 ± 1.2	56.3 ± 2.5	.80–.90	.20–.30
" -5	5	4	28.5 ± 1.3	55.5 ± 2.6	.50–.70	.20–.30
" -6	10		25.0 ± 1.2		.30–.50	
" -7	12		23.0 ± 1.1		.50–.70	
" -8	14		23.5 ± 1.1		.00[1]	
" -9	10		23.4 ± 1.1		.70–.80	
" -10	18		24.3 ± 0.9		.30–.50	
" -11	10	2	26.3 ± 1.2	57.3 ± 3.7	.10–.20	.50–.70
" -12	6	4	27.0 ± 1.6	55.9 ± 3.3	.70–.80	.70–.80
" -13	12		23.1 ± 1.3		.80–.90	
" -14	15		26.5 ± 1.1		.80–.90	
" -15	12	5	26.6 ± 1.1	55.4 ± 1.8	.30–.50	.00
" -16	11		23.5 ± 1.1		.90–.95	
" -17	6	6	23.9 ± 1.8	53.7 ± 2.2	.99	.10–.20
" -18	11		24.7 ± 1.1		.00[1]	
" -19	12		25.0 ± 1.2		.20–.30	
" -20	4		24.7 ± 1.9		.20	
" -22	16		25.8 ± 1.0		.10–.20	
" -23	6	4	24.1 ± 1.8	49.0 ± 2.7	.70–.80	.30–.50
" -24	15		24.6 ± 1.0		.30–.50	
	235	38	24.7 ± 0.3	54.2 ± 0.8		

[1]When two divergent progenies are omitted, P = .50–.70.

it had resulted from an authentic cross. Therefore, the observed ratio could not be attributed to the erroneous planting of an eversporting progeny instead of the intended F_1. Furthermore, the F_1 plants tested in the F_2 generation gave only 3 : 1 ratios, whereas only 1 : 1 ratios would be expected from an eversporting progeny.

In addition to the one F_1 progeny which gave an unexpected ratio of 1 : 1, four of them contained one or two exceptional doubles which also were of unquestionable hybrid origin. Both of these anomalous results may be accounted for by a phenomenon which will be discussed after considering the evidence for crossing over.

The two remaining hypothetical parental genotypes *Sl/S+* and *S+/S+* are theoretically not distinguishable from each other in the F_1 from crosses of their progeny plants with eversporting males since pure single F_1 progenies only are expected from each (table 5). However, in the F_2 generation following that cross different results would be expected for each. On the assumption that the

TABLE 5

Genotypic and phenotypic ratios expected from genetic tests on plants of three true-breeding single genotypes postulated to result from crossing over between the gene for single flowers and the lethal factor in a selfed eversporting plant.

True-breeding single genotypes	Genotypes expected in progenies from selfing indicated singles	Ratios expected from crossing indicated progeny plants with eversporting (*Sl/s+*) males	
		F_1 genotypes	F_2 phenotypes
			Sngl. : Dbl.
Sl/S+	1[1] *Sl/S+*	1 *Sl/s+* 1 *S+/s+*	1:1 3:1
	1 *S+/S+*	*S+/s+*	3:1
S+/S+	*S+/S+*	*S+/s+*	3:1
S+/sl	1 *S+/sl*	1 *S+/s+* 1 *sl/s+*	3:1 —
	1 *S+/S+*	*S+/s+*	3:1

[1]Slightly less than the indicated proportion of plants is expected for all genotypes containing *l*.

parent of progeny 5388 was of the genotype *S+/S+*, all of the plants in all F_1 progenies from individual plants of progeny 5388 crossed with eversporting males would be expected to be of the genotype *S+/s+* and to give 3 : 1 ratios exclusively in the F_2. Again, the data in table 3 show that this was not the case for the F_2 generation from such crosses. Therefore, *S+/S+* also is eliminated as a possible parental genotype for that progeny.

Finally, on the assumption that the genotype of the parent of progeny 5388 was *Sl/S+*, all of the plants in somewhat more than half of the F_1 progenies from the indicated crosses with eversporting males would be of the genotype *S+/s+* and would be expected to give only 3 : 1 ratios in the F_2. In each of the remaining F_1 progenies somewhat more than half of the plants would also be of the genotype *S+/s+* and would be expected to produce 3 singles : 1 double in the F_2 while somewhat less than half of the plants in the same progenies would be of the genotype *Sl/s+* and would be expected to produce approximately 1 single : 1 double in the F_2 with a consistent excess in the latter class (table 5).

From the total frequency of F_2 progenies in each percentage class (table 3) it is obvious that their distribution is bimodal with maxima at about 25% and 55% doubles. These maxima fit the expectation for genetic segregation of 3 : 1 and about 1 : 1 for singles versus doubles. The number of F_2 progenies placed in each of these genetic classes and the mean percentage of doubles for each class are given separately in table 4 for each tested plant in progeny 5388. The standard error values attached show that in no case is there a significant deviation of the mean percentage of doubles per family from genetic expectation for families in the 3 : 1 class. Half of the families in the 1 : 1 class deviate significantly in a positive direction from 50% doubles, but none were found to

deviate significantly from 55% doubles which is nearer the values 53–56% observed in eversporting lines (SAUNDERS 1911; FROST 1928; WINGE 1931).

Probability values from χ^2 calculated to test the homogeneity of the F_2 progenies placed in each class are also given in table 4 for the family from each of the 23 plants in the pure single progeny, 5388. It may be noted that in three instances among 31 the χ^2 test indicated a lack of homogeneity among the F_2 progenies classed together. The lack of homogeneity among 3 : 1 progenies from plants 5388-8 and 5388-18 was due to a low percentage of doubles in two of the progenies in each case, while the lack of homogeneity among 1 : 1 progenies from plant 5388-15 was due to an excess of doubles in one progeny. It should be borne in mind, however, that the percentage of doubles among progenies in the latter class is hypothetically in excess of 50% and is postulated to be augmented through the effect of adverse environmental factors (SAUNDERS 1911; KAPPERT 1937). Despite these minor discrepancies, it is apparent from the percentage of doubles (tables 3 and 4) in the F_2 progenies that essentially all of the tested F_1 plants from each of 14 out of 22 presumed pure singles in progeny 5388 produced only 3 : 1 ratios in the F_2, indicating a genotype of *S+/S+* for these 14 plants. Among the F_1 plants from each of the remaining 8 presumed pure singles in progeny 5388 more than half produced 3 : 1 ratios and less than half 1 : 1 ratios in the F_2. Also the mean percentage of doubles for progenies placed in the 1 : 1 class in the case of the totals and for most of the individual families was, as postulated, in excess of 50%. These facts demonstrate a genotype of *Sl/S+* for the 8 plants which gave these results. The fact that 14 plants in progeny 5388 were of the genotype *S+/S+* taken together with the fact that 8 were of the genotype *Sl/S+* provides the required evidence that the parent plant of that progeny was also of the genotype *Sl/S+*.

Reference has been made to the fact that singles (*Sl/s+*) in eversporting lines are smaller than their double sibs (*s+/s+*) in the seedling stage, ostensibly because of the effect of the lethal factor on the growth of the plant. This observation was verified in a survey preliminary to the present study which showed that the singles were significantly smaller than their double sibs in plant height and plant diameter taken at four months of age. Theoretically, therefore, F_1 progenies containing half *Sl/s+* and half *S+/s+* plants might be expected to exhibit slightly smaller means and greater variances for height and diameter of young plants than F_1 progenies containing only *S+/s+* plants. All of the F_1 progenies reported in this study were measured at 3 months of age. After the blossoming of their F_2 progenies it was possible to classify the F_1 data on plant size as shown in table 6. In both mean height and mean diameter of the plant the F_1 progenies in which half of the plants carried the lethal were smaller than those in which none of the plants carried the lethal. Also despite the fact that the greater variance would otherwise be associated with the greater mean, in this case the greater variance was associated with the smaller mean as postulated both for height and diameter of plant.

While none of these differences were statistically significant, they were all in the direction expected from the evidence that the parent plant of progeny

TABLE 6

Means and variances with their standard errors for plant height and diameter of F_1 progenies from individual plants of progeny 5388 × eversporting (Sl/s+) males.

Size measurement of 3-month-old plants	F_1 progenies in which all of the plants produced 3 singles : 1 double in the F_2		F_1 progenies in which half of the plants produced 3 : 1 and half produced 1 : 1 ratios in the F_2	
	Mean	Variance	Mean	Variance
Height (cm)	11.1 ± .26	2.9 ± .19	10.0 ± .36	4.1 ± .55
Diameter (cm)	16.6 ± .39	8.4 ± 1.16	15.3 ± .85	10.3 ± 1.97

5388 was of the genotype *Sl/S+*. That genotype, however, is here considered provisional until further evidence has been discussed.

Evidence for mutation of the S and l loci

In distinguishing between normal heterozygous and eversporting singles GOLDSCHMIDT (1913) and FROST (1915) as mentioned previously concluded that eversporting stocks either contained a lethal completely linked with the gene *S* for singleness or that the latter gene was so modified (S^l) in eversporting lines as to be itself lethal. The recovery of a normal heterozygous type by WINGE (1931) and a true-breeding single type by KAPPERT (1937) in eversporting lines was interpreted by them as evidence for crossing over between *S* and *l* as illustrated for the data obtained in the present experiment. On that interpretation the two genotypes *S+/s+* and *Sl/S+* would be expected with a relative frequency of 2 : 1 respectively (table 1). However, the mutation of $s \rightarrow S$ occurring on the *s+* chromosome would be expected to yield the same genotypes in the same ratio.

The exceptional results referred to in table 3 and similar exceptional results obtained by KAPPERT (1937) attest to the mutability of the *s* locus on the *s+* chromosome or to an irregular meiotic phenomenon either of which throws considerable doubt upon the alleged evidence for crossing over between *S* and *l*. Therefore, in the absence of evidence for mutation of either locus on the *Sl* chromosome specifically, it has not been shown conclusively that they are separate loci. Mutation of $l \rightarrow +$ would be expected to yield the same genotypes in the same ratio as the mutation $s \rightarrow S$, but available evidence as to the mutability of *l* is meager and clearly open to question. The reverse mutations $S \rightarrow s$ and $+ \rightarrow l$ would produce the unverifiable genotype *sl/s+* which also may be accounted for by crossing over as mentioned earlier.

While it has been provisionally demonstrated that the genotype of the parent plant of progeny 5388 was *Sl/S+*, one of the plants (5388-20) in that progeny had an undoubted genotype of *S+/s–* since it produced 19 singles : 18 doubles in the F_1 and only 3 : 1 ratios in the F_2 from an authenticated cross with an eversporting male (table 3). It is very unlikely that the presence of *s* in the genotype of plant 5388-20 was the result of a single accidental cross fertilization of its mother. Such a cross would have required pollen of the same geno-

type as the female with respect to flower color and would have had an equal likelihood of yielding an *Sl/s+* as the observed *S+/s–* plant. This evidence suggests that the mutation $S \rightarrow s$ was transmitted by one of the functioning gametes in the parent of progeny 5388. If *s* were carried by the pollen, it would give rise to two possible zygotes, *Sl/s+* and *S+/s+*. The former is excluded as a possibility in this case since it is expected to give 1 : 1 ratios in the F_2 rather than the 3 : 1 ratios observed. If *s* were carried by the egg, it would give rise to the two possible zygotes *S+/sl* and *S+/s+* both of which would exhibit 3 : 1 ratios in the F_2. It should be noted here for later reference that this possible mutation occurred in a plant of the genotype *Sl/S+* rather than *S+/S+*.

In progeny 5388 four additional plants all demonstrated by their F_2 ratios to be of the genotype *Sl/S+* gave exceptional doubles (table 3) in the F_1 from

TABLE 7

Proportion of double-flowered plants recovered from otherwise true-breeding singles selfed or crossed with eversporting males.

Source of tested plants	Genotypes of tested plants	Pollen used in test	Proportion of tested plants giving exceptional doubles	Percentage of eggs carrying s
Progeny from a selfed *Sl/S+* (*Sl/Ss+*)[1] plant	*Sl/s+* (*Sl/Ss+*)	*s+*	4/8	3.41
	S+/S+ (*Ss+/Ss+*)	*s+*	0/14	0
F_1 plants from the cross *Sl/s+* × *S+/S+*[2] (*Sl/s+* × *Ss+/Ss+*)	*Sl/S+* (*Sl/Ss+*)	*s+*	15/48	2.03
	S+/s+ (*Ss+/s+*)	*s+*	Not identifiable	—
F_2 plants from the cross *Sl/s+* × *S+/S+*[2] (*Sl/s+* × *Ss+/Ss+*)	*Sl/S+* (*Sl/Ss+*)	self	4/47	—
	S+/S+ (*Ss+/Ss+*)	self	0/66	0

[1]Revised genotypes are given in parentheses.
[2]Data from Kappert (1937).

authenticated crosses with eversporting males. Such doubles can be accounted for only by the occurrence of the mutation $S \rightarrow s$ in each of those plants or by a meiotic irregularity which simulates such a mutation. Among 176 plants in the F_1 progenies from *Sl/S+* plants six were doubles, indicating a possible mutation rate of 3.41% (table 7). None appeared in progenies of *S+/S+* plants. These facts strikingly coincide with observations made by KAPPERT (1937) who found that *Sl/S+* plants but not *S+/S+* plants obtained from the cross *Sl/s+* × *S+/S+* gave occasional doubles when selfed (table 7). He also recovered 2.03% of doubles from crosses of *Sl/S+* plants with eversporting males. In both instances he attributed the phenomenon to the mutation $S \rightarrow s$. However, he attributed to genetic crossing over the exceptional occurrence of the genotype *Sl/S+* in an eversporting line. This genotype, already referred to, was indirectly identified from capsule length differences. Since these two cases

more simply exemplify direct and reverse mutations there seems to be little reason to attribute them to different phenomena inasmuch as their observed frequency of occurrence is about the same. KAPPERT (*loc. cit.*) reports percentages of interchange for different lines ranging from 3.7% to .25%. Other instances are cited of higher percentages but these are attributed by him to lethals at other loci. From this evidence as to the apparent mutability of *S* it is clear that the recovery of the genotypes *Sl/S+* and *S+/s+* does not in itself constitute proof of crossing over between *S* and *l* nor proof that *S* and *l* represent different loci.

From his eversporting family, 82.3E, KAPPERT recovered a normal heterozygous plant which he attributed to interchange of the lethal, but in later generations from this plant he again obtained an eversporting line which he evidently attributed to a lethal mutation at a locus farther removed from *S* or to a chromosomal rearrangement or deletion. The association of the lethal effect in stocks with a chromosomal aberration was previously suggested by PHILP and HUSKINS (1931).

Evidence for the reduplication of the S locus

The attempt to disprove crossing over by demonstrating mutation of the two loci *S* and *l*, however, meets with difficulties. The *l* locus itself shows one of the distinguishing characteristics of a chromosomal aberration in being associated with nonfunctional pollen. Also the apparent mutation $S \rightarrow s$ is open to question because it is confined in every case to plants of the genotype *Sl/S+* and has not been recovered from *S+/S+* plants. As already stated, it occurred once in the parent of progeny 5388 which was of the genotype *Sl/S+* and six times exclusively in the *Sl/S+* plants, but not in the *S+/S+* plants of its progeny (table 3). It also occurred 15 times in F_1 plants of the genotype *Sl/S+* derived by KAPPERT (1937) from the cross *Sl/s+* × *S+/S+* (table 7). The F_1 sibs, in this case *S+/s+*, provided no measure of the apparent mutation frequency. It occurred again in selfed F_2 plants of the genotype *Sl/S+* but not in their selfed *S+/S+* sibs obtained by KAPPERT from the same cross.

The recovery of a double on selfing an *Sl/S+* plant requires an *s+* male gamete whereas an *sl* gamete, male or female, is not required. Hence, the apparent mutation $S \rightarrow s$ in addition to being restricted to the genotype bearing *l* appears to be further restricted to the homolog without *l*. These restrictions suggest that it involves a chromosomal aberration rather than a single gene mutation. A simple aberration that might account for the two crossover types recovered from eversporting plants and at the same time exhibit the observed restrictions would be the attachment of a fragment bearing *S* to the *s+* chromosome. Assuming that the *s+* chromosome normally carries a reduplication which places *s* between the two homologous segments, a crossover involving the reduplicated segment and the homologous portion of the *Sl* chromosome would cause the transfer of *S* to the *s+* chromosome so as to produce an *Ss+* gamete. The sister crossover gamete bearing the deficiency and presumably but not necessarily *l* would be nonfunctional.

From crosses with the trisomic, Slender as pollen parent FROST (1928, 1931) found " that in a double-thrower (eversporting) race functional pollen can carry a singleness locus provided it also carries a doubleness chromosome." PHILP and HUSKINS (1931), working with the variety Snowflake characterized by long meiotic chromosomes, found configurations of the A chromosome (i.e., the chromosome carrying the doubleness-singleness factors) which " seemed to imply that there are homologous regions at the end and in the middle of this chromosome." This implication was " strengthened by the observation that one of the A chromosomes alone can form a ring." It was further supported by " clear evidence of duplication of parts within the A chromosome of Crenate," a type which is trisomic for the A chromosome. These workers also state that " Apart from the more frequent observation of fragmentation, the abnormalities seen in the diploid Snowflake are . . . in general similar to those found in the short-chromosome races." The material involved in the present study is of the short chromosome type.

A reduplication on the *s+* chromosome is suggested here as a possible mechanism for obtaining the observed genetic results. Detailed cytological studies of the material involved may reveal a different mechanism. By whatever method obtained, a functional male gamete of the constitution *Ss+* fusing with a normal egg from the same eversporting plant would produce either an *Ss+/s+* or an *Sl/Ss+* plant. In their general breeding behavior plants of these genotypes would duplicate that of the postulated crossover genotypes designated as *S+/s+* and *Sl/S+* respectively. In addition an *Sl/Ss+* plant by infrequent transfer of the *S* fragment could produce exceptional *s+* gametes thus enabling it to exhibit in detail the breeding behavior observed for the parent of progeny 5388. In this case the transfer of the *S* fragment could be accomplished by a normal crossover in the region lying between *s* and the postulated reduplication.

The theoretical breeding behavior of a plant of the genotype *Sl/Ss+*, when subjected to the genetic tests which were applied to the parent of progeny 5388, is outlined in table 8. Only singles are expected from selfing such a plant except in the rare instance not shown in that table where two gametes having lost the *S* fragment unite. As already stated, KAPPERT (table 7) recovered exceptional doubles on selfing *Sl/S+* plants (here judged to have been *Sl/Ss+*) and attributed them to the fusion of two mutated gametes.

Plants of the noncrossover genotypes *Sl/Ss+* and *Ss+/Ss+* expected from selfing would exhibit the same breeding behavior in the F_1 and F_2 from crosses with eversporting males as that shown in table 5 for the progeny genotypes *Sl/S+* and *S+/S+* respectively, with the important exception, however, that the genotype *Sl/Ss+* by transfer or loss of the *S* fragment could yield exceptional doubles in the F_1 as did plants 3, 5, 12 and 23 in progeny 5388 (table 3). It should be noted again that all of these plants also contained the lethal factor as shown by the occurrence of both 1 : 1 and 3 : 1 ratios among their F_2 progenies. None of their homozygous *Ss+/Ss+* sibs without the lethal factor produced such exceptional doubles, and would not be expected to do so by a simple crossover.

TABLE 8

Genotypic and phenotypic ratios expected from genetic tests on a plant of the genotype Sl/Ss+ postulated to have resulted from irregular crossing over between the Sl and s+ chromosomes in a selfed eversporting plant.

Genotypes expected from selfing an *Sl/Ss+* plant	Ratios expected from crossing plants of indicated genotypes with eversporting (*Sl/s+*) males	
	F_1 genotypes	F_2 phenotypes Singles : Doubles
Noncrossover types		
1 *Sl/Ss+*	1[1] *Sl/s+*	1 : 1
	1 *Ss+/s+*	3 : 1
	– *s+/s+*[2]	—
1 *Ss+/Ss+*	*Ss+/s+*	3 : 1
Infrequent types from transfer of the *S* fragment		
1 *Sl/s+*	1 *Sl/s+*	1 : 1
	1 *s+/s+*	—
2 *Ss+/s+*	1 *Ss+/s+*	3 : 1
	1 *s+/s+*	—

[1]Slightly less than the indicated proportion of plants is expected for all genotypes containing *l*.

[2]Exceptional doubles from loss of the S fragment.

On selfing an *Sl/Ss+* plant (table 8) two additional genotypes, *Sl/s+* (the recovered eversporting type) and *Ss+/Ss+* would be expected infrequently as a result of transfer of the *S* fragment from the *Ss+* chromosome in formation of one of the gametes only. Both of these would exhibit 1 : 1 ratios in the F_1 as did plant 5388-20, but only the genotype *Ss+/s+* which occurs with twice the theoretical frequency of the other would give exclusively 3 : 1 ratios in the F_2 as did plant 5388-20.

It is postulated, therefore, that irregularity in crossing over between the *Sl* and *s+* chromosomes in eversporting stocks presumably occasioned by lack of structural homology may result in the reduplication of the *S* locus on the *s+* chromosome. Subsequent transfer of the *S* fragment from the *s+* chromosome can account for exceptional doubles produced by otherwise true-breeding single plants bearing *l*.

SUMMARY

Eversporting stocks are heterozygotes of the constitution *Sl/s+* in which *l* is a pollen-lethal factor and *s* is a recessive gene for the double-flowered form, which is sterile. They continue to produce 1 single : 1 double in successive generations. An exceptional true-breeding single plant, however, was recovered from an eversporting line. This plant was tested for the presence of *l* in its genotype by crossing 24 of its progeny with eversporting males (pollen all *s+*).

Among 23 F_1 progenies obtained, 18 consisted of only single-flowered plants, 4 contained exceptional doubles and one consisted of 19 singles : 18 doubles. All

F_2 progenies from the last F_1 consisted of 3 singles : 1 double only, indicating a genotype of *S+/s–* for the parent tested in that cross.

Fourteen of the remaining 22 parents tested in the crosses produced only 3 : 1 ratios in the F_2, indicating a genotype of *S+/S+* for these 14 parents.

Among the F_1 plants from each of the now remaining 8 presumed pure single parents (including the 4 which produced exceptional doubles in the F_1) more than half produced 3 : 1 ratios and less than half 1 : 1 ratios in the F_2, demonstrating a provisional genotype of *Sl/S+* for each of these 8 parents.

Genotypes of *S+/S+* for 14 and *Sl/S+* for 8 of the plants in the pure-single progeny demonstrated a genotype of *Sl/S+* for the parent of that progeny. That genotype may have resulted from crossing over between *S* and *l* in the eversporting (*Sl/s+*) line.

However, since exceptional doubles occurred in F_1 progenies from plants having the lethal factor but not in their sibs without the lethal, and since the plant which segregated 1 : 1 in the F_1 occurred also in the progeny of a plant having the lethal factor, it is suggested that plants of the provisional genotype *Sl/S+* actually carried the *s+* chromosome with an *S* fragment attached (*Sl/Ss+*).

It is concluded that the exceptional true-breeding single plant (*Sl/Ss+*) recovered in an eversporting line (*Sl/s+*) arose by irregularity in crossing over which produced an *Ss+* gamete. The subsequent infrequent transfer of the *S* fragment in that parent and in four of its progeny plants resulted respectively in the observed 1 : 1 F_1 progeny and exceptional doubles in four additional F_1 progenies.

Results obtained by other workers but differently interpreted by them were found to substantiate these conclusions.

ACKNOWLEDGMENTS

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CHIASMATA AND CROSSING OVER IN A DICENTRIC CHROMOSOME IN WHEAT[1]

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ONE important concomitant of the now generally accepted partial chiasmatypy theory of crossing over is that every chiasma constitutes a crossover. Unfortunately, as DARLINGTON (1937) points out, there has been very little opportunity to test this concept. Meiotic chromosomes of Drosophila are too small for an accurate determination of chiasma frequency, and maize is about the only other organism in which enough linkages have been established to insure that nearly the full genetic length of each chromosome is represented in the map. In maize DARLINGTON (1937) reported satisfactory agreement between the published map lengths of the various chromosomes and the values predicted on the basis of his own counts of chiasma frequency, and BEADLE (1932) found a good correspondence between map length and the frequency of chiasmata in a particular segment of chromosome 9 of maize. In both cases, however, the genetic and cytological data were derived from different experiments, and crossover values in maize are known to be variable. The experiments of OEHLKERS (1940) and ERNST (1938) showing similar effects of environmental changes on chiasma frequency and crossover frequency in Oenothera and Antirrhinum, respectively, argue strongly for a one-to-one correspondence between chiasmata and crossovers. COOPER (1944), on the other hand, has shown that some apparent chiasmata have no relation to crossing over but are merely "conjunctive segments."

The transmissible dicentric chromosome of wheat described by SEARS and CÂMARA (1950, 1952) appears to provide an additional opportunity for testing the concept of chiasmata-crossover correspondence. This chromosome consists of a long arm, a main or strong centromere, an intercentromeric region, a second weak or possibly defective centromere, and a minute arm (fig. 1). The arm lengths have the approximate ratio 30:10:1. The secondary centromere is evidently inactive in bivalents during the first division of meiosis; in other divisions it seems to behave like any other centromere.

When two dicentrics pair, a chiasma in the intercentromeric region, if it is a crossover, should lead to bridge formation at AI, as shown in figure 1. Bridges of the type expected do occur, and their frequency is readily determi-

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nable. The frequency of chiasmata in the intercentromeric region at MI can also be determined with reasonable accuracy. Chiasmata in this region cannot be distinguished from those that may occur in the minute end piece, but this minute arm is so short that the number of chiasmata occurring there may be assumed to be negligible. Also, if two or more chiasmata occurred in the intercentromeric region, it is doubtful that they could be distinguished from a single chiasma; but this region is short enough that two chiasmata there must be very infrequent. Furthermore, with two chiasmata, only the two- and four-strand doubles would fail to lead to bridges.

SEARS and CÂMARA (1952) found that the frequency of chiasmata in the intercentromeric region did not differ greatly from the number of bridges observed in anaphase I (13 percent). However, they used estimates rather than

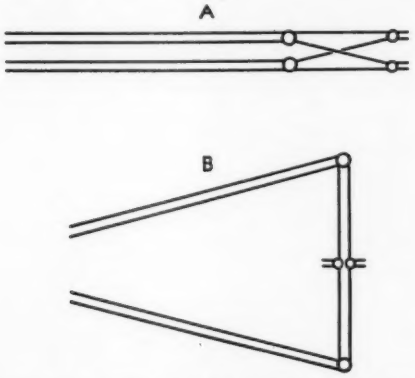


FIGURE 1.—Diagram of a pair of dicentric chromosomes with a chiasma in the intercentromeric region, at (A) pachytene and (B) anaphase I.

precise counts of chiasmata, and their preparations, which were made from spikes fixed entire, did not allow the analysis of a high proportion of cells.

In the spring of 1951 one plant with two dicentrics was grown. The anthers were fixed individually, and meiosis was studied in seven preparations, each of a single anther. In the fall of 1951 five more plants with two dicentrics were obtained, and a total of 43 single-anther preparations were studied. The results are presented in table 1. The data showed good homogeneity when tested for differences between plants, and the results may therefore be summed up as follows.

METAPHASE I

At metaphase I the dicentric chromosomes were unpaired in 21.1 percent of the cells that were analyzed. In these they lay off the plate as in figures 2 and 3 of SEARS and CÂMARA (1952). In 15 cells or 1.3 percent they were paired but lay off the plate and acted like the above univalents, both centromeres being active (*ibid.*, fig. 15). In the remaining 880 cells (or 77.5 percent)

TABLE 1

Meiotic behavior of a pair of dicentric chromosomes.

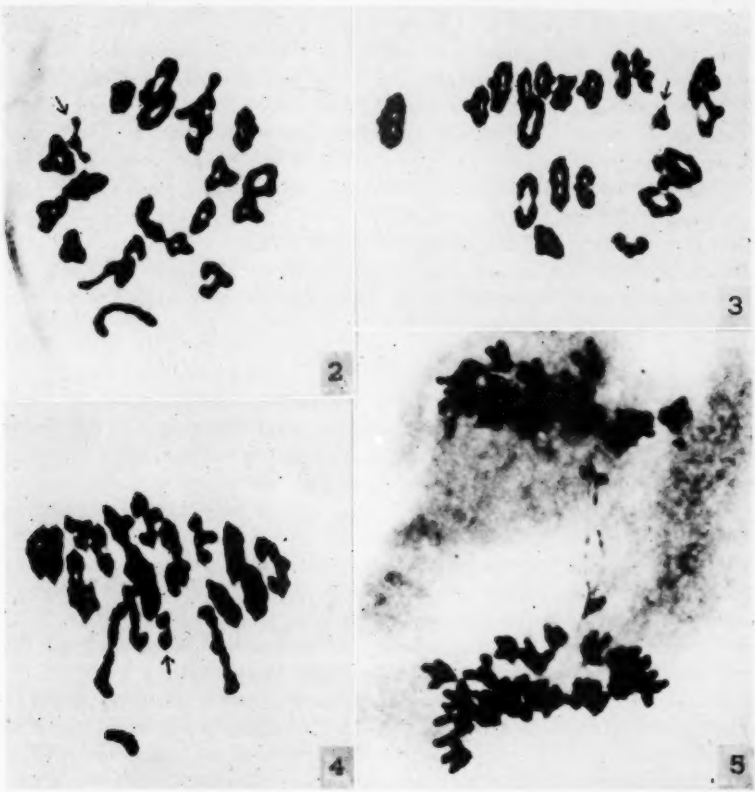
Plant No.	No. anthers	Metaphase I						Anaphase I		
		Unpaired	Paired, but acting as univalents	Xma in each arm	Xma in short arm only	Xma in long arm only	Unidentified	Bridge	No bridge	Unclassified
1	7	8	0	19	1	34	....	5	29	....
2	6	19	2	22	0	30	181	13	107	9
3	7	30	2	36	2	87	52	9	51	12
4	15	100	7	93	7	197	259	20	183	39
5	6	35	1	47	0	98	80	6	82	7
6	9	48	3	83	8	116	306	7	100	13
Totals	50	240	15	300	18	562	878	60	552	80

the dicentrics were paired, lying on the plate with the appearance of a normal bivalent in as much as only the strong centromere appeared active. The dicentric bivalent most frequently occurred as a rod bivalent with one chiasma in the long arm, and was easily recognized by its typical shape (fig. 2). Less frequently it was seen as a small ring bivalent with one chiasma in each arm (fig. 3). Very rarely, in only 18 cells or 1.6 percent of the total, it had a single chiasma in the short arm (fig. 4). Thus in 862 cells or 75.8 percent the dicentrics had a chiasma in the long arm, and in 28.0 percent of the total cells there was a chiasma in the short arm. As will be shown, this is a much higher frequency of short-arm chiasmata than would be expected from anaphase data. The question therefore arises as to whether the 878 unanalyzed metaphases, amounting to 45 percent of the total, may have included an unduly high proportion of the cells without short-arm chiasmata. This is very unlikely; in fact, the reverse is almost certainly true. Identification of dicentric ring bivalents was only possible in well-spread cells, where all 21 pairs could be studied and none discerned to be a dicentric rod bivalent. On the other hand, poorly spread cells were frequently classifiable if they contained a dicentric rod pair, because of the characteristic, easily recognizable appearance of this bivalent. Thus the unanalyzed metaphases consisted of the unfavorable cells less a considerable number in which the dicentric rod was identified. Since there is every reason to believe that the unfavorable cells were a random sample with respect to pairing behavior, it is very likely that the unanalyzed group had a higher proportion of bivalents with short-arm chiasmata than did the population as a whole.

ANAPHASE I

Anaphase I was studied and a count of dicentric bridges obtained (table 1). The characteristics of the dicentric bridge permit easy identification; it is double, and frequently the small knob or bump consisting of the minute end can be seen (fig. 5). It can be readily distinguished from the univalent dicentric bridge that would be expected from about 1 percent of unpaired dicentrics (SEARS and CÂMARA 1952). Only about 0.4 percent of the anaphases in this material would be expected to have such univalent bridges. Bridges con-

cerned with other chromosomes than the dicentrics occasionally occurred in this material, and some fragments also occurred. A few anaphases, about 12 percent of the total, too early for bridge determination, had to be excluded from the analysis.



FIGURES 2-4.—First meiotic metaphase showing paired dicentrics. × 960. FIGURE 2.—Bivalent with an interstitial chiasma in the long arm. FIGURE 3.—Ring bivalent with one chiasma in each arm. FIGURE 4.—Bivalent with a chiasma in the short arm.

FIGURE 5.—First meiotic anaphase showing the double dicentric bridge. The knobs near the center presumably represent the minute arm beyond the secondary centromere. × 960.

Only 60 cells, or 9.8 percent of the total 612 anaphases studied, had dicentric bridges. This represents a great paucity of bridges as compared to the 28.0 percent chiasmata in the intercentromeric region that should result in such bridges. This finding will be discussed in detail below.

ANAPHASE II

Both anaphase I and anaphase II were studied to check the possibility that a chiasma in the intercentromeric region might result in early breakage at the secondary centromere without bridge formation in anaphase I. Such breakage would give rise to derivative chromosomes lacking the secondary centromere and to fragments consisting mainly of the minute arm and the secondary centromere. No such derivative chromosomes or fragments were observed. Fragments were found in six cells, but in several of these the dicentric was complete and unchanged. Apparently the occasional fragments found in this material were entirely unrelated to the dicentric chromosome.

Following its inclusion in a telophase I nucleus, each dicentric would be expected to behave as described by SEARS and CÂMARA (1950, 1952) for undivided univalent dicentrics, namely with both centromeres active but with the two centromeres of each chromatid usually directed toward the same pole. This appeared to be the case, since in only 5 out of 62 second anaphases was the secondary centromere seen to pull (ineffectively) toward the opposite pole from the primary centromere.

DISCUSSION

Although prophase analysis of bivalent dicentrics was not possible, metaphase figures indicated that the two chromosomes had in almost all cases been paired throughout their length. Thus the secondary centromeres had presumably been paired with each other; yet these centromeres ordinarily showed no poleward activity during the first division. Fifteen bivalents were found in which the secondary centromeres were active, but in each of these the secondaries had clearly not been paired with each other but with the corresponding primary centromere (cf. SEARS and CÂMARA 1952). Hence there was no exception to the rule that the secondary centromeres showed no poleward activity during first division when they had been paired with each other.

Since there are secondary centromeres in rye (KATTERMANN 1939; PRAKKEN and MÜNTZING 1942) and maize (RHOADES and VILKOMERSON 1942) which are only active at a particular stage of meiosis, the question might be raised whether the secondaries in the wheat material are actually capable of first-division activity. The wheat secondary centromere is clearly active at this stage in univalent dicentrics, however, and in the 15 exceptional bivalents just mentioned; so there seems little doubt that it is capable of activity in the ordinary bivalents also.

So far as is known, the secondary centromeres behave normally at all other divisions, although they are weaker than the primary when opposed to it. It appears, therefore, that the secondary centromere lacks something necessary to insure the separation of the two halves of a bivalent but not required at mitotic divisions. When a secondary pairs with a primary centromere, as it evidently does in a univalent dicentric, the primary is able to initiate not only its own poleward movement but also movement of the secondary centromere. This evidence that something more is required of a centromere at meiosis than

at mitosis may be of significance in connection with problems of chromosome mechanics and the structure of the centromere. It is possible that a study of the behavior of certain derivative chromosomes will provide further information of value in this regard.

It seems clear that the bridges observed are formed in the manner indicated in figure 1—that is, by failure of chiasmata in the intercentromeric region to terminalize beyond the secondary centromeres. The bridges are double, as expected, and frequently a lump can be detected at the center of the bridge which finds ready interpretation as being the minute arm. Bridges formed in another way could occur here, in as much as SEARS and CÂMARA (1950, 1952) suggest that the intercentromeric region may contain a reversed duplication. Crossing over in this region reversely paired would result in bridges, but these would usually be single and would be accompanied by fragments.

The data thus show a real discrepancy in the frequency of chiasmata in the intercentromeric region as compared with the frequency of anaphase bridges formed. As has already been pointed out, this discrepancy cannot be due to early breakage. The disjunction without bridge formation must come about in some other way.

The possibility that most of the chiasmata counted as intercentromeric are in reality in the minute end beyond the secondary centromere, and therefore resolvable without bridge formation, has not been excluded. However, because of the small size of the minute end, this is believed to be unlikely. The relatively high frequency of such chiasmata necessary to account for the paucity of anaphase bridges would indicate a special preferential localization of chiasmata in this end. It seems unwarranted to postulate such localization, particularly since the relative frequencies of chiasmata in the long and short arm of this chromosome agree well with expectation based on their lengths.

If sister secondary centromeres could separate at AI, then terminalization of intercentromeric chiasmata could proceed, and there would be no bridges. In normal bivalents homologous centromeres pass to opposite poles at first division, while sister centromeres do not separate until second division. In univalents, on the other hand, sister centromeres separate at first division, although considerably later than do the bivalent halves. The secondary centromeres under investigation resemble centromeres of univalents in that the undivided centromere shows no poleward activity. This suggests the possibility of further resemblance, namely, in the separation of sister centromeres at the first division. The occurrence of bridges in 9.8 percent of first anaphases would then be attributed to the secondary centromeres having been active in about one-third of the bivalents with intercentromeric chiasmata, with consequent failure of terminalization across these active centromeres. No such activity of secondary centromeres was ever seen, but it could have occurred without being detected in the bivalents with intercentromeric chiasmata, since these were usually tightly paired. However, since activity of secondary centromeres was not seen in bivalents with only long-arm chiasmata, where it could scarcely have gone unobserved, it probably did not occur in the bivalents with inter-

centromeric chiasmata either. The only obvious explanation for limitation of secondary centromere activity to the latter type of bivalent would be that activity of the secondaries might be a consequence of their pairing with each other. Although this pairing would favor chiasma formation in the neighboring intercentromeric region, there seems no reason to suppose that it would invariably result in chiasma formation in this short segment.

Thus there seems to be no satisfactory way of reconciling the present data with the theory that every chiasma represents a crossover. However, of the three possibilities suggested—namely, early breakage, localization of chiasmata in the minute arm, and premature separation of sister secondary centromeres—only early breakage has been entirely excluded. The data do not, therefore, constitute critical evidence against the partial chiasmatypy theory. They do suggest, however, the desirability of further work with this and similar material.

SUMMARY

A transmissible dicentric chromosome in wheat has centromeres of unequal strength. In plants with two dicentrics, these usually pair, and no activity of the weaker centromere can then be detected. In 28.0 percent of the cells at MI of microsporogenesis there apparently was a chiasma in the intercentromeric region. This should have led to an equivalent frequency of bridges at AI, according to the generally accepted theory that every chiasma represents a crossover, but only 9.8 percent of AI cells had bridges. This discrepancy is not considered as critical evidence against the theory, however, since other explanations are not entirely excluded.

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EVIDENCE FOR SISTER-STRAND CROSSING OVER IN MAIZE[1]

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CROSSING OVER, the exchange of chromatin between homologous chromosomes, is of utmost importance in genetic studies. Although little is known about the mechanism which is responsible for this exchange, a number of facts on this subject have been fairly well established: (1) Crossing over is produced by breakage and reunion of broken ends (even on BELLING's hypothesis, breakage and reunion have to be postulated to explain 3-strand and 4-strand doubles). (2) The position of the exchange is exactly between homologous regions of the chromosomes. (3) Crossing over occurs at prophase of the first meiotic division. (4) Only 2 of the 4 chromatids are involved in any one crossover. (5) The occurrence of one crossover decreases the probability of another occurring in its vicinity—the phenomenon of interference. It has generally been assumed that there is little or no sister-strand crossing over. This

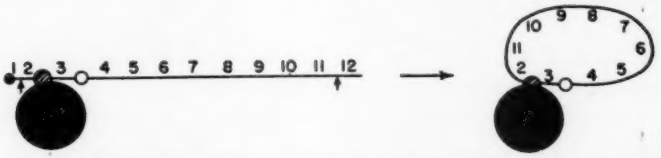

FIGURE 1.—Diagrammatic representation of the formation of the ring chromosome following breakage in the distal regions of chromosome 6. The nucleolus is represented by the large black circle and the centromere by the clear oval. The shaded area represents the nucleolar organizer. The arrows indicate the probable sites of X-ray-induced breakage. (Reprinted through courtesy of the American Naturalist.)

conclusion has been drawn from studies in Drosophila with attached-X chromosomes (BEADLE and EMERSON 1935) and with the Bar locus (STURTEVANT 1925, 1928; MULLER and WEINSTEIN 1933). However this is still an open question. The pioneering work of MCCLINTOCK (1938, 1941b) on dicentric double-sized ring formation and the evidence to be presented in this paper suggest that crossing over does occur between sister chromatids. Such an event can be detected only with ring chromosomes with the possible exception of "unequal" crossing over in sister rod chromatids giving rise to duplicated segments.

In a previous paper (SCHWARTZ 1953) the behavior of a large ring in maize involving almost the whole of chromosome 6 was discussed. Gametophytes possessing nine chromosomes plus the ring are viable even though deficient for the terminal regions of chromosome 6 (fig. 1). Crossing over in the hetero-

[1] Work performed under USAEC Contract No. W-7405-eng-26.

zygote between the ring and its homologous rod may result in bridges in both anaphase I and anaphase II. Only crossovers in the long arm of the chromosome are being considered since those in the short arm between the centromere and the nucleolar organizer are very infrequent (McClintock 1941b). A

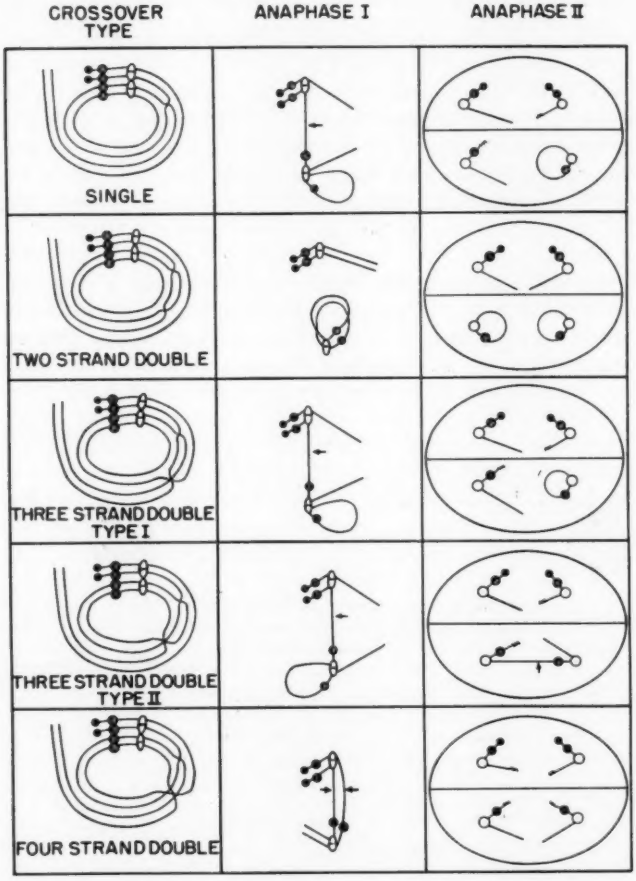

Figure 2.—Anaphase configurations resulting from crossing over between the ring and its homologous rod chromosome. (Reprinted through the courtesy of the American Naturalist.)

——~~ = broken chromosome end ——→ = position of breakage

single or a 3-strand double crossover (type I), in which the same ring chromatid is involved in both exchanges, gives a single bridge in AI only (fig. 2). Both 2-strand double crossovers and non-crossovers result in normal disjunction without bridge formation. A 3-strand double of the second type (II), in which the same rod chromatid is involved in both exchanges, gives a single

bridge in both AI and AII. A 4-strand double gives a double bridge in AI. These bridges are not associated with fragments.

Two of the double crossover classes can easily be distinguished—type II 3-strand doubles and the 4-strand doubles. Equal frequencies of double bridges in AI and single bridges in AII would therefore indicate the absence of chromatid interference.

RESULTS AND INTERPRETATION

Four plants heterozygous for the ring and a rod chromosome were used in this study. The frequencies of the various anaphase configurations observed are listed in table 1. The anaphase II data are given in terms of daughter cell pairs which show a single or double bridge in one of the two cells. This frequency was calculated in the following manner. All cells in AII were counted regardless of whether they were found singly or in pairs. Of these cells 166 showed single bridges, 47 double bridges (from dicentric rings), and 737 no bridges. Type II 3-strand doubles and sister-strand crossovers in the ring give rise to bridges in only one of the two daughter cells, the other being normal. Therefore, 213 cells (166 + 47) must be subtracted from the 737 and the remaining 524 divided by 2 (i.e., 262 as given in table 1) to determine the number of daughter cell pairs which lacked bridges.

TABLE 1

Meiotic anaphase configurations observed in plants heterozygous for a ring and a rod.

	Anaphase I				Anaphase II (daughter cell pairs)			
	Single bridge	Double bridge	No bridge	Total	Single bridge	Double bridge	No bridge	Total
Number	368	81	171	620	166	47	262	475
Percent	59	13	28	100	35	10	55	100

There are two important facts which should be pointed out in the data. First, the frequent occurrence of double bridges (dicentric rings) in AII. These would not be expected from single and double crossovers and only from 1/16th of the triple crossovers. Second, the frequency of single AII bridges.

It is apparent from table 1 that single bridges in AII are much more frequent than double bridges in AI, approximately a threefold difference. Can this difference be interpreted as being due to negative chromatid interference; i.e., the participation of one strand in a crossover enhances the probability that it will be involved in the second? This might appear to be the case, since anaphase configurations resulting from 3-strand doubles where one of the strands is involved in both crossovers, are more frequent than those arising from 4-strand doubles where each strand is involved in only one crossover. However, on this basis the frequency of 2-strand doubles should be even greater than 3-strand doubles. As the data indicate, this is not true. Only 28 percent of the AI cells showed no bridges and some of these are presumably due to non-crossovers.

If the 35 percent single bridges in AII are due entirely to type II 3-strand double exchanges, an equal frequency of type I might be expected. Thus, 70 percent of the AI cells would show 3-strand double crossing over and if one then added the frequency of double bridges, no bridges, and single bridges due to single crossovers, the total would come to much more than 100 percent. It seems clear therefore that negative interference cannot explain the data and that the frequency of single bridges in AII is not a true measure of the frequency of type II 3-strand doubles. The frequent occurrence of triple crossovers between the ring and the rod is unlikely and it would not account for the ratio of observed bridge configurations.

The high frequency of single bridges in AII can be accounted for by sister-strand crossing over (fig. 3). The term " sister-strand crossing over " as used

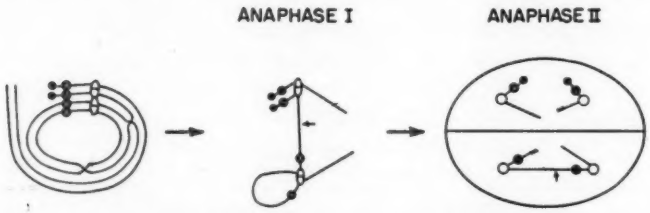

FIGURE 3.—Anaphase configurations resulting from a single non-sister crossover associated with a sister-strand crossover between the ring chromatids.

here refers simply to an exchange of chromatin between sister chromatids and does not imply any relationship to non-sister-strand crossing over in the time and manner of its occurrence, interference, etc.

There are four classes of anaphase configurations which result from double non-sister crossovers between the rod and ring (fig. 2). These are (1) single bridge in AI only, (2) single bridges in AI and AII, (3) double bridge in AI, and (4) no bridge in AI. Considering only non-sister crossing over, these classes would arise from 3-strand doubles of type I, 3-strand doubles of type

TABLE 2

*Theoretical ratio of anaphase configurations resulting from double non-sister-strand crossovers associated with sister-strand crossing over.**

No. sister-strand crossovers	Single bridge AI only	Single bridge AI and AII	Double bridge AI	No bridge AI		
				No bridge AII	Double ring AII	Total
0	1	1	1	1	0	1
1	1	1	1	0.5	0.5	1
2	1	1	1	0.5	0.5	1
3	1	1	1	0.5	0.5	1
Many	1	1	1	0.5	0.5	1

*This table is based on the assumption of no chromatid interference and was calculated by following the consequences of the four non-sister double crossover types in all possible combinations with varying numbers of sister-strand crossovers.

II, 4-strand doubles, and 2-strand doubles, respectively. The proportions of these four classes are not changed by the occurrence of sister-strand crossing over (table 2). Regardless of the frequency of the latter, whether it be one or 10 per bivalent, the proportions of these classes would remain the same as those resulting from double non-sister crossovers alone. If the number of sister-strand crossovers per bivalent is high, an odd number occurring in any one region will appear as a crossover and an even number as a non-crossover. Thus, the frequency of single bridges in AII resulting from double non-sister crossovers associated with sister-strand crossing over should still be equal to the frequency of AI double bridges, i.e., 13 percent.

Another way by which single bridges in AII can arise is by a single non-sister crossover associated with sister-strand crossing over. An odd number of sister crossovers will give rise to a single bridge in AI and AII. An even number or no sister crossovers will result in a single bridge in AI only. It is not possible to estimate from the data the number of sister-strand crossovers which occur per bivalent except that it must be one or more to account for the high frequency of single AII bridges. One sister crossover together with a single non-sister crossover will give 50 percent bridges in AI only and 50 percent bridges in AI and AII if there is an equal probability that it will occur in the ring or in the rod, since only sister crossovers in the ring result in AII bridges. Likewise, a large number of sister-strand crossovers per bivalent will give 50 percent of each type if there is equal probability of an odd or an even number occurring in the rod and ring.

Single bridges were observed in 59 percent of the anaphase I configurations. These result from the single and half of the double non-sister crossovers. If there is no chromatid interference, 26 percent of the anaphase I cells would possess single bridges due to 3-strand double crossovers, since the frequency of 4-strand doubles was 13 percent. As stated previously, the frequency of single bridges in AI only and the frequency of single bridges in AI and AII resulting from double non-sister crossovers should each be equal to the frequency of AI double bridges. This leaves 33 percent AI single bridges due to single non-sister crossovers. As a result of sister-strand crossing over, half of these or 16.5 percent will also form single bridges in AII. Thus we can calculate that 29.5 percent single AII bridges would be expected on the basis of one or many sister-strand crossovers per bivalent, i.e., 16.5 percent from the single non-sister crossovers and 13 percent from the double non-sister crossovers. The observed value was 35 percent. This difference is not statistically significant.

In somatic mitoses the frequency of sister-strand crossing over in a ring chromosome can be determined by measuring the frequency of double-sized rings. The probability of two such crossovers occurring per ring and thus effectively cancelling each other is slight in view of the low frequency of double rings. This calculation cannot be made for meiosis where the ring and rod synapse, since only a portion of the sister-strand crossovers give rise to double rings. Dicentric rings arise from sister-strand crossing over in bivalents

that had either no non-sister or double non-sister crossovers. The anaphase I cells showing no bridges, which result from double non-sister crossovers, should be equal in frequency to the cells showing AI double bridges—13 percent (table 2). Since the observed frequency of no bridge configurations in AI was 28 percent, this leaves 15 percent which did not have a non-sister crossover.

On the hypothesis of a large number of sister crossovers per bivalent, the frequency of double rings resulting from double non-sister crossovers would be one half the frequency of double bridges, or 6.5 percent (table 2). Also, half the bivalents not having a non-sister crossover, 7.5 percent, would give double rings. Thus, the expected frequency of double-sized rings would be 14 percent; the observed frequency was 10 percent.

The frequency of double rings in mitotic anaphases is quite low. McCLINTOCK (1938) counted only 8 percent double rings arising from a large ring which involved most of chromosome 2. The ring used in this study is somewhat smaller and gave 4.5 percent double rings, 57 out of 1275, as measured in meristematic root tissue. This striking difference between the meiotic and mitotic rates may offer a clue to the mechanism of sister-strand crossing over.

DISCUSSION

MATSUURA (1940, 1948) has presented evidence that in Trillium the two chromatids in each chromosome are coiled in a relational spiral system at early meiotic metaphase I and in a parallel spiral system at late metaphase I. He has shown that the chromosome can undergo this change in coiling only by breakage and subsequent reunion of the chromatids at each half coil. He has used this mechanism to explain legitimate crossing over by postulating that the paired chromatids of each chromosome are non-sisters in two-thirds of the cases. There are many objections to such a hypohesis. The frequency of crossing over would be much higher than normally found. Also, crossing over should be reduced in the distal ends of a chromosome since the twisted chromatids will be able to separate without breakage in that region, as has been pointed out by Matsuura. However, in maize one of the most striking cases of non-correlation between cytological and crossover distances occurs in the very distal end of chromosome 9 where yg_2 and *dt*, which are cytologically very close, show 7 percent crossing over (RHOADES 1945).

Furthermore, this theory cannot explain the formation of anaphase bridges with fragments from heterozygous paracentric inversions. MATSUURA has recognized this difficulty and in a later paper (1950) made the unlikely suggestion that there is no correlation between the occurrence of paracentric inversion loops at pachytene and bridges with fragments at anaphase.

MATSUURA's mechanism for the parallelization of the spiral system could, however, account for sister-strand crossing over if we assume that the paired chromatids in each bivalent are sister strands. The number of such crossovers per bivalent would be high, roughly equal to the number of half coils. As has been pointed out, this is consistent with the data. This hypothesis would also

explain the low mitotic rate of sister-strand crossing over since, in " somatic " mitosis, each of the two chromatids of a chromosome usually takes an independent spiral system from the beginning of its development (MATSUURA 1940).

Misdivision of the centromere is an alternative hypothesis to sister-strand crossing over which should be considered. Such a mechanism can be ruled out in a number of ways. The correlation of the frequency of dicentric ring formation with the size of the ring (MCCLINTOCK 1938) is strong evidence against the production of double-sized rings by misdivision of the centromere. Double-sized rings arising from centromere misdivision would differ in the arrangement of the genes on the ring from those arising by sister-strand crossing over, and hence result in a different pattern of mosaicism (fig. 4). Endosperm mosaics resulting from the instability of a ring chromosome involving the short arm of chromosome 9 and carrying C (colored aleurone) and Wx (starchy endosperm) were analyzed (SCHWARTZ, unpublished). If the double-sized rings arise by centromere misdivision, twin sectors of colorless starchy and

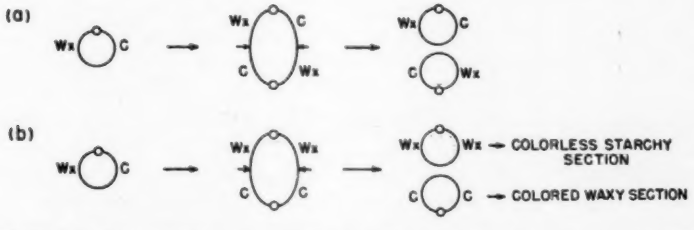

FIGURE 4.—Diagrammatic representation of endosperm mosaic pattern resulting from (a) dicentric ring arising by sister-strand crossing over, and (b) dicentric ring arising by misdivision of the centromere.

colored waxy tissue should be frequent. These would result from breakage of both bridge strands between C and Wx. No such twin sectors were found.

In order to explain the high frequency of single bridges in anaphase II by this mechanism, one would have to postulate that the undivided centromere of a dyad in which two of the arms are connected misdivides frequently while the centromere of a dyad with four free arms divides normally (fig. 5). It is difficult to visualize how such a modification in the morphology of the dyad would affect the plane of division of the centromere. Moreover, in the course of the breakage-fusion-bridge cycle described by MCCLINTOCK (1941a), chromosome configurations are formed by the fusion of broken ends of sister chromatids which are similar to the dyads described above in that the centromere is undivided, and two of the arms are free and two are joined. Normal division of the centromere results in such a dicentric chromatid. Misdivision of the centromere would result in the formation of a centric ring and a centric rod. The breakage-fusion-bridge cycle, which results in a mosaic phenotype when in the proper genetic background, persists only in the gametophytic and endosperm tissue. The mosaicism is not carried over into the sporophyte since

the broken ends heal in the embryo and the cycle is halted (McCLINTOCK 1941a). If rings are formed by centromere misdivision, the mosaic pattern should be evident in the sporophyte because of the unstable behavior of ring chromosomes.

Fusion of the broken ends of an AI double bridge might be thought of as an alternative hypothesis to explain the high frequency of single bridges in AII. However, McClintock has shown that following the breakage of an AI double bridge fusion did not occur in the telophase I nucleus. In our study no cases were found where each daughter cell of a pair contained an AII bridge.

The evidence which is most frequently cited against the occurrence of sister-strand crossing over is the frequency of homozygosis in attached-X females of Drosophila. If the distribution of chiasmata among the four strands at meiosis is at random, there are six possible combinations of these four strands and the frequency of homozygosis cannot exceed 16.7 percent. If only non-sister strand

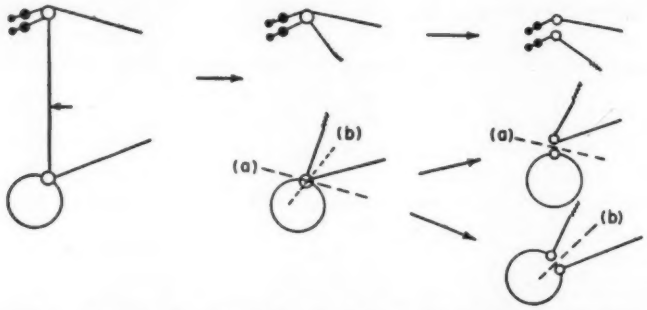

FIGURE 5.—Schematic diagram of alternative hypothesis involving misdivision of the centromere. (a) Represents normal plane of division of the centromere; (b) represents misdivision of the centromere.

crossing over occurs, the expected homozygosis value is 25 percent when one exchange occurs, 12.5 percent for two exchanges, 18.75 percent for three exchanges, etc. (SAX 1932). The finding of homozygosis frequencies significantly higher than 16.7 percent was interpreted as proving that little or no sister-strand crossing over occurs (BEADLE and EMERSON 1935). However, as these authors have pointed out, this evidence rules out only sister-strand crossing over which is equivalent to non-sister crossing over and shows chiasma interference. If the two crossover types are independent, as has been proposed in this paper, arising by different mechanisms and occurring at different times in the meiotic division, the maximum frequency of homozygosis expected from a combination of both sister- and non-sister-strand crossing over would remain at 25 percent.

From the study of the stable X^{c1} ring chromosome in Drosophila, MORGAN (1933) concluded that no sister-strand crossing over occurs. Her argument was as follows: Sister-strand crossing over in the ring would result in dicentric double-sized rings which would be eliminated in oogenesis. In females hetero-

zygous for the ring and a rod X chromosome this elimination would result in a decrease in the frequency of gametes carrying a non-crossover ring chromosome. The fact that equal ratios of non-crossover ring and non-crossover rod gametes were produced is certainly strong evidence against the occurrence of sister-strand crossing over in the X^{c1} ring. However this does not warrant a generalization regarding such crossing over in other organisms. If dicentric ring formation is used as the criterion, the evidence is strongly in favor of sister-strand crossing over in maize. It is an established fact that dicentric double-sized rings do arise from ring chromosomes in maize (McCLINTOCK 1938, 1941b).

The behavior of ring chromosomes in Drosophila is quite different from that in maize and presents an extremely complicated and little understood picture. The X^{c2}, In(1)w^{rc} ring is highly unstable and gives rise to frequent gynandromorphs (BRAVER and BLOUNT 1950). The X^{c1} and X^{c2} rings are comparatively stable but the stability of the X^{c2} ring has been shown to be greatly influenced by environmental conditions (BROWN and HANNAH 1952). No stable ring chromosomes have been reported in maize.

In discussing the occurrence of those mutations at the Bar locus in Drosophila which were not associated with crossing over between forked and fused, STURTEVANT (1925, 1928) placed little emphasis on the possibility of explaining these mutations by unequal sister-strand crossing over. He was of the opinion that these exceptional cases arose as a result of contamination. In a similar study involving the compound A^b locus in maize, LAUGHNAN (1952) reported 8 percent exceptional cases where the separation of the α and β components of the locus was not associated with crossing over. It was possible to rule out contamination in these experiments. LAUGHNAN lists three possible explanations for these exceptional cases: (1) unequal sister-strand crossing over, (2) mutation of the β component to a null form, and (3) deficiency of the β component. The last two hypotheses are not very satisfactory. As LAUGHNAN pointed out, the exceptional cases are identical with those isolated from A^b by crossing over and are limited in the time of their occurrence to meiosis. If the non-crossover exceptions involve deficiencies of β, the deleted segments are very small including neither α nor sh_2 which are very closely linked with and situated on either side of β. While the A^b exceptional cases are not in themselves strong evidence for sister-strand exchange, they are at least suggestive and can be used as supporting evidence for such a form of crossing over.

SUMMARY

A large discrepancy in the expected ratio of anaphase configurations resulting from crossing over between a large ring involving chromosome 6 and its homologous rod chromosome is interpreted as due to sister-strand crossing over. The data indicate that at least one sister-strand crossover occurs per bivalent. An hypothesis is presented for the mechanism responsible for such crossing over based on Matsuura's observation on the parallelization of the spiral system in meiotic metaphase.

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GENETIC ANALYSIS OF SEVEN NEWLY DISCOVERED MUTANT ALLELES AT LOCUS T IN THE HOUSE MOUSE[1]

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ONE of the eventual goals of genetical research is to elucidate the relationship between four unknown but discoverable characteristics of the genetic material. These might be listed as: 1) the type of change to which one genetic locus or area is subject (mutation); 2) the structural character of that locus or area; 3) the kinds of developmental processes that are affected by changes in that localized material; and 4) the role which a locus with such properties may play in adaptive responses to the environment and thereby its probability of incorporation in some future species or varietal genotype. If we were suddenly to gain an understanding of these relationships the genetical millenium would have arrived, and we should, as investigators, temporarily join the unemployed. But we know that such an event is not at hand, for precise knowledge of the relationship between these primary attributes is not likely to develop from the study of any one species or any single line of attack. The goal is too distant a one to give us the practical directions we need in charting our way among the detailed technical studies which form the indispensable raw material for synthesis. Yet if we bear in mind what the end is, we shall at least be influenced in our choice of means, and choose to follow up, in the more modest ways in which experimental studies must be circumscribed, those problems and materials which offer some hope of making progress in these four problems, which show signs even now of having a single direction.

It is probably some such thoughts which have encouraged us to persist over a period of years in the study of material which many people consider refractory and slow but which to us presents fascinating immediate problems and almost daily surprises on the way toward a more ambitious and distant goal. The house mouse, as bred in captivity for hundreds of years, is as everyone knows, good for breeding experiments. Four or five generations a year, if not equal to the daily or bimonthly generation period of other useful animals and

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[2] It would not have been possible for us to maintain the breeding operations and observations on which work of this sort depends without the devoted help of a number of students and assistants of whom we should especially like to thank RUTH SEGAL and SHIRLEY KAMELL.

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plants, is at least pretty good for an animal with warm blood which must suckle its young for three weeks and keep up a nearly continuous activity in confinement. We have always had great respect for one other quality of the mouse which underlies its usefulness for genetical research, its innate variability, which is of the same order as that which marks the animal toward which much mouse research is aimed, that is, man. But until we began to study one character, and particularly one area of one chromosome intensively, we had not realized how great is its potential variability. This character was simply tail length and form, in which the effects of some thirty-odd individual mutations have been observed, and of these more than half have been found to be concentrated in one small area of one chromosome.

The discovery of the first mutant in this area was made by DR. DOBROVOLSKAIA-ZAVADSKAIA in 1927, and she generously gave us not only stock of the original mutation, which was marked by a short tail, but of three other changes which turned out to be alleles of the first. Analysis of these alleles (CHESLEY and DUNN 1936; DUNN and GLUECKSOHN-SCHOENHEIMER 1939) led to the establishment of the balanced lethal system which is the key to the detection of further changes at this locus. We have been studying these changes both genetically and developmentally since 1936. KOBOZIEFF and POMRIASKINSKY-KOBOZIEFF (1939a and b) have independently reached the same interpretation concerning the first two mutations. In the present report we shall deal only with the genetically analyzed mutational changes found in one of the balanced lethal lines. This one has been called Line 29 and has been shown to breed true to taillessness in the manner shown below.

Tailless × Tailless

T/t^1	×	T/t^1
T/T	T/t^1	t^1/t^1
dies	viable	dies
10th day	Tailless	<5th day

Actually we have observed at birth 3462 offspring from matings of $T/t^1 \times T/t^1$. Of these 3321 were regular (completely tailless) and 130 had short stumps or even short tails up to one-half of normal. Some of the latter have been tested and shown to be T/t^1; most of them are probably phenotypic variants of this genotype. But the remaining 11 had tails of normal length and these are probably valid exceptions. We succeeded in analyzing seven of these and each of these proved to have the genetic constitution t^1/t^n, t^n being a new allele at this locus. Of the four which could not be analyzed one died before testing, two were males which proved to be sterile, and one, a male, produced three small litters (he was probably quasi-sterile) of which the few descendants were lost in the F_2.

A conspectus of the seven which were analyzed in shown in table 1, together with the original mutation t^1 and a probable recurrence of it, t^2, found in

another balanced line (19). All of the t^n alleles agree in several important attributes. 1) All produce taillessness in compound with T and are recessive, in tail effect, in $+/t^n$. 2) All fail to show recombination with T or with t^1; this is the definition of their allelism. 3) Each new allele produces a normal-tailed compound t^1/t^n, although the viabilities of these compounds are not equal. This is the condition which permitted the detection of new changes. 4) All show normal disjunction in females T/t^n; while some males of each T/t^n compound show nonrandom segregation of T and t^n.

The alleles differ and several can be individually characterized by one or more of several properties. 1) Five are lethal when homozygous, and of these four can be distinguished by the stage and the manner in which the lethal effect occurs; while the fifth, not yet studied embryologically, differs from all the others in its extreme segregation ratio in male heterozygotes (see table 8).

TABLE 1

Some effects of seven new mutant alleles at locus T which produce taillessness in combination T/t^n, compared with those of the balanced line T/t^1 in which they arose.

Allele	Homozygote	Characters chiefly affected	Segregation ratio T/t^n ♂ $t^n : T$
T	Lethal 10th day	Notochord; allantois	1
t^1	Lethal < 5th day	Preimplantation	> 1
t^2			
t^4	Lethal 8th day	Early differentiation	< 1
t^9	Lethal 9th day	Early differentiation	< 1[1]
t^{12}	Lethal ?	Not studied	≫ 1
t^3	Viable	Normal	± 1
t^7	Viable	Normal	1
t^8	Viable	Normal	1
t^{11}	Viable	Normal	± 1

[1] 1 male out of 7 gave high ratio ($115t^9 : 49T$).

2) All of the t^n lethals, when in compound with T, in males, segregate in some ratio which departs from the normal 1 : 1 but in different directions in different lethals. Thus in T/t^1 and T/t^{12} males the t^1 and t^{12} sperm outnumber those with T, sometimes by wide margins, while in T/t^4 and T/t^9 the reverse is generally, but not uniformly the case ($T/+$ shows normal segregation). 3) The relative viabilities before birth of some of the compounds of lethal alleles are different, thus t^1/t^{12} has very low viability while t^1/t^4 and t^1/t^9 have much higher viabilities before birth. Variation in viability of compounds of the same alleles was found in the case of t^1/t^0 (t^0 occurred independently of t^1) and shows that factors at other loci are also involved in viability effects. In this case also characteristic abnormalities were found in some of the t^0/t^1 embryos (DUNN and GLUECKSOHN-SCHOENHEIMER 1943). Future work may reveal specific effects of t-alleles and of other factors in compounds.

The four viable alleles are not immediately distinguishable since all produce normal or nearly normal tails in the homozygotes, and the segregation ratios

TABLE 2

Tailless line 3 (T/t^3).

♀ Parents ♂		Normal	Brachy	Tailless
Tailless × tailless	$T/t^3 \times T/t^3$	197		474
Normal × tailless	$+/+ \times T/t^3$	343	401	1
Tailless × normal	$T/t^3 \times +/+$	71	72	1
Brachy × tailless	$T/+ \times T/t^3$	79	84	101
Normal × normal	$t^3/t^3 \times t^3t^3$	315		
Brachy × normal	$T/+ \times t^3/t^3$	154	4[2]	178
Normal[1] × Brachy	$t^3t^3 \times T/+$	273		213

[1]Phenotypic variants probably T/t^3.
[2]From cross of $T/t^3 \times T/t^4$.

of their male compounds T/t^n are not seriously disturbed. Yet even amongst the viable alleles there are indications of some individual differences. In most of the male compounds t^1/t^n which have been tested, fertility is either absent or impaired, but probably to different degrees in different combinations. Thus most t^1/t^3 males are sterile with a few quasi-sterile, while t^1/t^7 males have

TABLE 3

Tailless line 4.

♀ Parents ♂		Normal	Brachy	Tailless
Tailless × tailless	$T/t^4 \times T/t^4$			334
Normal × tailless	$+/+ \times T/t^4$	522	788	1*
Tailless × normal	$T/t^4 \times +/+$	166	156	
Brachy × F_1 normal	$T/+ \times +/t^4$	370	184	171
F_1 normal × Brachy	$+/t^4 \times T/+$	30	20	17

*Phenotypic variant of $T/+$.

higher fertilities (DUNN 1952). Before reliable distinctions of this sort amongst the viables can be made, the different stocks must be made otherwise isogenic and this has not yet been completed.

These judgments are of course tentative and preliminary. The breeding data on which they rest are set out in tables 2–8. The indications at present are that many of the changes found in the T/t^1 balanced stock, and analyzed, are new and different from others. It is as yet too soon to say whether all of the effects

TABLE 4

Tailless line 7.

♀ Parents ♂		Normal	Brachy	Tailless
Tailless × tailless	$T/t^7 \times T/t^7$	30		76
Normal × tailless	$+/+ \times T/t^7$	150	161	
Tailless × normal	$T/t^7 \times +/+$	22	21	
Brachy × tailless	$T/+ \times T/t^7$	120	117	126
F_1 normal × Brachy	$+/t^7 \times T/+$	51	26	24
Brachy × normal	$T/+ \times t^7/t^7$	34		32

TABLE 5

Tailless line 8.

♀ Parents ♂		Normal	Brachy	Tailless
Tailless × tailless	$T/t^8 \times T/t^8$	99	1*	202
Normal × tailless	$+/+ \times T/t^8$	166	167	3#
Tailless × normal	$T/t^8 \times +/+$	92	94	1
Brachy × tailless	$T/+ \times T/t^8$	59	45	36
Tailless × Brachy	$T/t^8 \times T/+$	28	37	28
Brachy × F_1 normal	$T/+ \times +/t^8$	38	9	16
F_1 normal × Brachy	$+/t^8 \times T/+$	66	29	25
Brachy × normal	$T/+ \times t^8t^8$	8		11
$t^8t^8 \times t^8t^8$		13		

*Phenotypic variant of t^8/t^8.
#One tested was phenotypic variant of $T/+$.

by which alleles appear to differ, such as segregation ratio and degree of male sterility in compounds, are properties peculiar to each allele rather than of the genetic background of the stocks in which they have been studied. But some distinctions, such as lethality and viability and the stage and manner of lethal action, are surely properties primarily of the individual alleles.

TABLE 6

Tailless line 9.

♀ Parents ♂		Normal	Brachy	Tailless
Tailless × tailless	$T/t^9 \times T/t^9$			175
Normal × tailless	$+/+ \times T/t^9$	184	212	
Tailless × normal	$T/t^9 \times +/+$	97	91	
Brachy × tailless	$T/+ \times T/t^9$	73	87	62
Tailless × Brachy	$T/t^9 \times T/+$	10	7	11
Brachy × F_1 normal	$T/+ \times +/t^9$	53	25	18
F_1 normal × Brachy	$+/t^9 \times T/+$	70	52	26

Assuming for the present that each exception found represents a different change in locus *T*, what can be said about the nature of the changes? Are they mutations in the broad sense of the word as meaning novel changes in a gene or chromosome, or are they, like many of the changes in Oenothera which were described as mutations, also as in the latter case rare recombinations due to

TABLE 7

Tailless line 11.

♀ Parents ♂		Normal	Brachy	Tailless
Tailless × tailless	$T/t^{11} \times T/t^{11}$	14		50
Brachy × tailless	$T/+ \times T/t^{11}$	41	49	64
Tailless × Brachy	$T/t^{11} \times T/+$	7	15	8
F_1 normal × Brachy	$+/t^{11} \times T/+$	18	7	8
Normal × tailless	$t^{11}/t^{11} \times T/t^{11}$	37		39
Brachy × normal	$T/+ \times t^{11}/t^{11}$	9		18

crossing over within the balanced lethal system? Categorical answers cannot be given but evidence at present rather favors the mutation hypothesis. The detectable results of crossing over in $T+/+t^1$ for example should be wild type gametes ++ and these in combination with regular gametes T and t^1 should produce exceptions $T/+$ and $t^1/+$. These have never been detected. Ordinary recombination should produce repeatedly the same types of crossover gametes whereas each of the exceptions analyzed has proved to be different. GRÜNEBERG (1952) has suggested that since t^1 is probably an inversion, crossing over within it might give rise to duplications of some loci and deficiencies of others and thus the compounds of different lethal changes should be viable. Without nearby chromosome markers this hypothesis cannot be tested at present. It would require that some duplications and deficiencies should be viable since we have four viable alleles at the T locus. If it is an inversion it must be very short (JAFFE 1952); consequently crossing over within it should tend to produce short deficiencies or duplications which repeat themselves, that is, the same allele should arise repeatedly but not new inversions. We do not yet know whether the new lethal alleles like the older ones t^1 and t^0 give evidence of

TABLE 8

Tailless line 12.

♀ Parents ♂		Normal	Brachy	Tailless
Tailless × tailless	$T/t^{12} \times T/t^{12}$			94
Brachy × tailless	$T/+ \times T/t^{12}$	32	9	30
Normal × tailless	$+/+ \times T/t^{12}$	146	13	
F_1 normal × Brachy	$+/t^{12} \times T/+$	42	14	15
Tailless 29 × tailless 12	$T/t^1 \times T/t^{12}$	26		256
Tailless 12 × tailless 29	$T/t^{12} \times T/t^1$	10		150
F_1 normal × Brachy	$t^1t^{12} \times T/+$	62		45

being inversions by the suppression of crossing over (DUNN and CASPARI 1945). At least one of the new viable alleles (t^3) does take part in recombination in the *T-Ki* region (DUNN and GLUECKSOHN-WAELSCH, in press).

The frequency of these changes must be very high. The exact ratio is difficult to estimate. We have found a minimum of 7 and probably 11 changes within a few years and amongst only 3500 offspring observed from $T/t^1 \times T/t^1$. All of the exceptions tested had the composition t^1/t^n and several of these compounds have less than normal viability, so that some of them may die as embryos and escape detection.

Most of those detected occurred in related individuals of one family line of a Line 29 T/t^1 stock. The t^4 exception was found in the inbred descendants (four generations of brother-sister matings) of the pair which produced t^3. The original t^1/t^4 exception (a female) was mated to $T/+$ and both tailless (T/t^1 and T/t^4) and normal-tailed ($+/t^1$ and $+/t^4$) offspring were tested. One normal son crossed to $T/+$ had 13 tailless offspring which lived to be tested by T/t^1. The results of these tests are shown in table 9.

There is no doubt that the father of these 13 animals which bred like T/t^1 was himself $+/t^1$, but in the T/t^1 offspring (or in the T/t^1 animals by which

they were tested) three new *t*-alleles arose. The extensive number of test offspring observed was of course in part responsible for the result; but 3 new changes in 437 observations is a rate within this family line which must be higher than in the general population of T/t^1.

Similarly, the origins of t^{10}, t^{11}, and t^{12} are connected; t^{10} was found in the fifth brother-sister generation following a cross of T/t^1 to $T/+$. The mother of the t^1/t^{10} exception was mated to a brother of the exception, and the resulting group of a brother and his sisters produced 84 tailless and one normal which proved to be t^1/t^{11}. The same brother of the t^1/t^{10} exception was mated to his sister, and amongst the grandchildren of these one normal-tailed exception was found, and from a sibling of this t^{12} was isolated.

It is perhaps coincidence that the T/t^1 families in which the exceptions were found were the only ones which regularly produced the phenotypic variants of

TABLE 9

Tests of 13 tailless offspring from a cross of Brachy T/+ females by ♂ 19157 +/t^1, a son of the original mutant t^1/t^4.

Animal No.	Progeny from test cross with T/t^1		Diagnosis
1	33 tailless		T/t^1
2	37 tailless		T/t^1
3	44 tailless		T/t^1
4	99 tailless	1 normal (= t^1/t^8)	T/t^1
5	33 tailless		T/t^1
6	18 tailless		T/t^1
7	57 tailless		T/t^1
8	8 tailless	1 normal (= t^1/t^7)	T/t^1 ?
9	20 tailless		T/t^1
10	24 tailless		T/t^1
11	15 tailless	1 normal (= t^1/t^9)	T/t^1 ?
12	20 tailless		T/t^1
13	29 tailless		T/t^1
Total	437		

T/t^1 known as stump tails or short tails and also sometimes produced offspring with urogenital abnormality resembling those with the mutation *ur* which was first found in one of these lines.

These related occurrences suggest some sort of inherited instability or some chainwise connection as though the first change had touched off a succession of other ones. The connection in the seven cases analyzed however was always through t^1 since the new alleles did not arise from each other but always from T/t^1. Inbred lines of $T/+$ (Brachy) on the other hand which we have bred since 1930 appear to be stable. The occasional tailless animals found in these lines have always proved when tested to be $T/+$, that is, they are phenotypic modifications of the Brachy phenotype. This does not mean necessarily that the changes in the T/t^1 line occur in the t^1 chromosome. In the one viable allele tested for crossing over suppression, the chromosome with t^8 had lost the inversion, or at least the crossover suppressor associated with t^1. The changes may of course be induced in the T chromosome by the presence of t^1.

One fact of special interest was noted in two cases. Although the occurrence of a normal-tailed exception heralded the presence of a new allele in all cases, in t^3 and t^{12} the new tailless line was not obtained solely from the exception. In the case of t^3, reasons were given (DUNN and GLUECKSOHN-WAELSCH 1951) for assuming that the change occurred more than once in related individuals, and in at least one of these it probably occurred before the last meiotic division since several mutated gametes were recovered from an individual known to be T/t^1. This may have been the case also with t^{12}, for the exception, a male, was sterile and a new allele was recovered from two tailless siblings of the exception. Each of these proved to be T/t^{12} by giving normal-tailed offspring when tested by T/t^1 from the standard line. Two other sibs similarly tested gave 37 and 34 tailless offspring. It is not unlikely that in the mouse, mutation in premeiotic stages will prove to be less uncommon than in insects and that this is not peculiar to the *T*-locus but applies to some other loci as well (W. S. RUSSELL 1951).

There is no evidence that factors at other loci are responsible for the instability. Although t^3 appeared only one generation after an outcross of T/t^1, the other alleles occurred in descendants of five to eight generations of brother-by-sister matings so that introduction of other factors from outcrosses is probably not responsible.

Moreover, the presence of t^1 is not an essential condition for the instability since we have also found normal-tailed exceptions in the inbred balanced Line A, T/t^0, two in 1942 and two in 1952. None of these exceptions, due to poor viability and sterility, has given rise to lines which could be analyzed.

Finally, as shown recently (DUNN and MORGAN 1952), *t*-alleles have also been found in populations of wild mice from widely different localities.

This opens up a host of problems concerning the function of this locus in the wild which we hope can be studied by us and others interested. One great question is what permits *t*-alleles to be maintained, possibly above equilibrium values, in the wild. In addition to the factors of heterozygote advantage and mutation pressure which have been investigated in other materials, the first *t*-allele isolated from the wild segregates from heterozygotes in ratios far exceeding 1 : 1. Our first wild males $+/t^w$ produced about six t^w gametes for each + gamete. This may more than compensate for its homozygous lethal effect; and this may provide a clue to the persistence of lethal mutations and to the unusual segregation mechanism as of some adaptive significance.

Thus in spite of the high frequency with which such genetic changes arise, it seems most reasonable at present to treat them as "mutations," although "mutation" should not exclude the type of two-break changes which MULLER has called rearrangements. The large number of them detected in the T/t^1 lines, although it certainly implies high mutability, is also a reflection of the ease of detection and identification in such lines. The balanced lethal systems T/t^n, where t^n is a lethal, are as it were traps baited to catch mutants of this type; that is mutations to alleles which show fairly high viability in compound with the old allele. Mutants with low viability in compound will tend to escape this trap.

Those mutations from one viable allele to another cannot of course be detected by this balanced lethal method. We are testing another method for detecting these which may prove to be too cumbersome, that is, the different degrees of interaction in male compounds t^n/t^x in respect to sterility and quasi-sterility. We know for example that t^3 and t^7, both viable, show quite different interactions in this respect with t^1; t^1/t^3 males are sterile or nearly so; t^1/t^7 may have nearly normal fertility (DUNN 1952).

DISCUSSION

What now can be said, in the light of the data given above, about the general questions in the introduction? It is evident at the least that three of the primary characteristics of the genetic material can be identified and profitably studied at locus T in the mouse: the types of mutative change, the kinds of developmental processes they give rise to and the role of the locus in adaptive responses. Concerning these traits some evidence has been obtained and some suggestions about relations among them have emerged. The fourth trait, the structural character of the locus, is still unknown, and in the absence of the essential cytogenetic evidence no conclusive or adequate hypothesis relating the four traits can be formulated.

It is clear that the locus is quite mutable both in the balanced condition T/t^1 in which 11/3500 changes to t^n were noted, in T/t^0 (4/2000) and in wild populations, four out of five of which were found to contain $+/t^w$ individuals, t^w being any allele of T found in the wild. The likelihood that the changes found in T/t^1 populations are merely recombinations in balanced lines is reduced by finding that similar changes are common in wild populations. The mutability of the locus appears not to depend on any particular combination of alleles, or even upon the previous presence of a change at this locus, since distant populations contain diverse t^w alleles.

At least three different types of alleles have arisen as a result of these changes: (1) the dominant lethal type T recorded by DOBROVOLSKAIA (1927) and independently by CARTER and PHILLIPS (1950), and which also may have occurred in the stocks of DUBOSQ (1922); (2) recessive lethals such as t^0, t^1, t^4, t^9 and t^{12}; (3) recessive alleles such as t^3, t^7, t^8 and t^{11} which are viable and normal-tailed when homozygous. Each of the last two types has also been found in wild mice. Two of the lethals (t^0, t^1) prevent recombination in the immediate neighborhood of T, suggesting that they contain sectional changes, possibly inversions; while at least one viable allele, t^3, is not a crossover suppressor. All act as alleles with each other t^0/t^3, t^4/t^7, etc. It is probable then that both sectional changes and point mutations arise with some frequency in the same area but the relation of these changes to the physical conditions in the chromosome is unknown.

Concerning the kinds of developmental processes affected by these changes, we know a good deal more. As pointed out previously (GLUECKSOHN-WAELSCH 1951) " changes in most of the genetic factors in Chromosome IX (near locus T) lead to abnormalities of derivatives of the notochord-mesoderm material and to phenomena traceable to an absence of functioning or to abnor-

mal functioning of this same material." The specific deviations from normal development associated with the several types of change at locus *T* are closely related to each other and in one case to those at a neighboring locus.

In the case of *T*, both notochord and somites are abnormal from early stages and death occurs at about 11 days. In the homozygote t^0/t^0 which dies shortly after implantation (5th day) no mesodermal structures at all are formed; in the case of t^1 we know only that the homozygote does not reach the stage of implantation, but we do not know when or how the zygote dies. In t^4/t^4 the development of the archenteron, which in the mouse is involved in the formation of notochord-mesoderm, is disturbed. Preliminary studies of t^9 homozygotes which die at 8–9 days have revealed abnormalities involving duplications of parts of the neural system which indicate a relationship with the homozygote *Ki Ki*, a mutation at a nearby locus. Inductive phenomena traceable to abnormalities in function of notochord-mesoderm material could well be responsible for the duplications in t^9/t^9 embryos. The similarity in homozygous effect of *Ki* with t^9, which is allelic with *T* while *Ki* is not an allele of *T*, seems very significant. The fundamental effects of the lethal members of the *t*-series studied up to now (*T*, t^0, t^1, t^4, t^9) all fit the assumption that the region of chromosome IX in which they together with *Ki* are located plays an important part in the normal functioning of the notochord-mesoderm material. The embryological studies have shown very clearly that while all *t*-type mutations studied have a common focus, each lethal can be distinguished by its developmental effect from each other one (*T*, t^0, t^1, t^4, t^9) proving that each lethal has originated in a different mutative event. This locus or area, because of its effect on structures of primary developmental importance, must have an important adaptive role. Its mutability may be correlated with its high developmental activity, and both may be related to the kind of material of which this region is composed. Since mutants, many of them lethal, are retained in heterozygous form in wild populations, the locus in heterozygous condition may make some positive contribution to development and viability. We know also that changes at this locus affect the segregation ratio $T : t^n$ and $+ : t^n$ in spermatogenesis. The mechanism which provides for an excess of lethal-bearing sperm, for example, t^1 or t^{12}, may thus have a definite adaptive value.

In sum, although it can not now be specified in detail how the mutability, developmental processes, and adaptive role of this locus are related, we believe our work has shown them to be interdependent, and specific experiments can now be planned to elucidate the mechanism of interdependence.

SUMMARY

Evidence from breeding experiments is presented in summary form indicating the occurrence by mutation in the balanced lethal line T/t^1 of three new lethal alleles (t^4, t^9, t^{12}) and four alleles (t^3, t^7, t^8, t^{11}) which are viable in homozygous form; these seven were found in about 3500 offspring from $T/t^1 \times T/t^1$ observed at birth together with four additional exceptions which were lost before genetical analysis could be made. Comparison of the breeding

behavior of these and review of evidence on developmental studies led to conclusions that locus T is 1) highly mutable; 2) concerned (together with a neighboring locus Ki) in the functioning of the notochord-mesoderm in the early embryo; 3) connected with some adaptive function which leads to retention of *t*-mutants, even when lethal, in wild populations. Relations between these characteristics of the locus are discussed.

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AN ANALYSIS OF VARIABILITY ARISING THROUGH RECOMBINATION[1]

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THE existence of variation among the individual members of a population has been recognized for centuries; the biological importance of this variation has begun to be understood only in the past few decades. Formerly the type specimen was regarded as representing the species; now it is realized that adequate representation is obtainable only through a series of specimens. Population geneticists of the 1920's and 1930's were active in studying the genetic component of this variability. CHETVERIKOV (1926, 1927), TIMOFEEFF-RESSOVSKY and TIMOFEEFF-RESSOVSKY (1927) and DUBININ (1934) demonstrated the existence of mutant genes in natural populations of Drosophila. BAUR (1924) and TIMOFEEFF-RESSOVSKY (1934) detected spontaneous and induced mutations with extremely minute phenotypic effects. Combined, this information amounted to the discovery of a vast store of partially concealed genetic variability upon which a species may draw for evolutionary changes. A simplified, symbolic illustration of this concept represents the majority of surviving adults in a population as *AABB...*, *AaBB...*, *AABb...* and *AaBb....* The interbreeding of these types also produces *aaBB...*, *aaBb...*, *Aabb...*, *AAbb...* and *aabb...* individuals but the inclusion of these among the breeding portion of the population depends upon the degree to which they are opposed by selection. The repetitive formation of these individuals in each generation, however, guarantees that these genotypes are available should the nature of selective forces change in their favor.

This theory, still the prevailing one, explains the existence of gametic variability beneath a uniform phenotypic façade. If, for example, *aa* and *bb* in our scheme represent lethal genotypes, surviving individuals carry *A* and *B* in at least the heterozygous condition and possess the invariable phenotype *AB*. Recent data indicate that this is an oversimplification—real differences exist between different individuals of a population. Following the studies on striking and constant differences indicative of balanced polymorphism made by FISHER (1930), FORD (1945) and TIMOFEEFF-RESSOVSKY (1940), WRIGHT and DOBZHANSKY (1946) demonstrated conclusively that in spite of the absence of morphological differences individuals of *D. pseudoobscura* heterozygous for two different gene arrangements of the third chromosome have higher adaptive values than individuals homozygous for either of these third chromosome

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arrangements alone. The possibility that this is merely a subtle manifestation of homozygosis for recessive genes among structural homozygotes was eliminated through the further study of chromosomes from different geographical localities; in the latter case the structural heterozygotes are no longer superior to both homozygotes (DOBZHANSKY 1950). The " co-adaptation " of chromosomes of one arrangement to those of another, with the resultant production of structural heterozygotes of superior adaptive values, is an intra-population phenomenon. Actually, CROW (1948) has pointed out that theories of heterosis based simply upon the concealment of deleterious recessive genes by their dominant alleles fail to account for differences in adaptive values of the magnitudes reported in WRIGHT and DOBZHANSKY's original paper.

A further indication that the genetic situation within populations is not as simple as suggested by the " *A–a–B–b* " scheme is found in the phenomenon of " iso-alleles." STERN and SCHAEFFER (1943), working with the gene cubitus interruptus (*ci*) in *D. melanogaster,* tested a number of mutants against several " wild type " alleles derived from different sources. Each of the wild types analyzed showed its own unique pattern of reaction of dominance in relation to different temperatures. This suggests that the symbol ci^+ that represents the wild type is really a catch-all for an unknown number of distinct and variant systems.

In the development of predictions on the " *A–a–B–b* " system, it is generally assumed that selection coefficients are small and that interactions between different genes are absent. The first assumption (besides appearing sensible) allows the use of calculus in the mathematical manipulations and permits further assumptions about approaches to equilibria. The second assumption allows calculations to be made for classes of genes (lethals, semilethals, subvitals) without concern as to the permanence of the classes as other postulated changes occur. WRIGHT (1931), it is true, has pointed out that the behavior of a gene in a population depends upon its reaction—and the reactions of every one of its alleles—to the entire array of genotypes in which it is found. Since a complete analysis of so complex a system is an obviously impossible experimental or mathematical task, simplifying assumptions must be introduced into the calculations. Until critical experiments have been made, it should be remembered that these mathematical models are working hypotheses awaiting confirmation. It is now known that selective forces may be much greater than those frequently postulated. It is also known that seemingly minor environmental changes can alter—even reverse—selective forces observable at any one time. Striking genic interactions have been recorded time and again. It seems reasonable, then, to question the concept of smooth, asymptotic approaches of mutant genes to their equilibrium frequencies in populations of long standing.

Further information regarding the genetic structure of populations became necessary to explain the following facts—the results of a detailed analysis of several experimental populations: The average viability of individuals heterozygous for randomly chosen second chromosomes frequently increased concurrent with a demonstrable accumulation of " deleterious genes "; the via-

bilities of individuals homozygous for second chromosomes of different populations was not correlated with the viabilities of individuals heterozygous for random combinations of these same chromosomes; and, observed viabilities seemed to be largely characteristic of particular chromosomal combinations rather than of the chromosomes entering into the combinations. These results, seemingly not in harmony with simple modifications of the "A–a–B–b" scheme, suggested instead a rapid action of selection on a rather fluid genetic complex.

THE ARGUMENT

Assume that a particular chromosome of a species carries a number of loci and that at several of these loci there are two alternative alleles *A–a*, *B–b*, etc. Assume, further, that selection within a given population favors individuals of the phenotype *AB*..... The alleles *A* and *B* will be the common alleles in the population at their respective loci, for example, while the alleles *a* and *b* will be rare; the chromosomes *AB*.... will be common, *Ab*... and *aB*... will be relatively scarce, and *ab*... will be very rare. If a sample of chromosomes were taken from different individuals of such a population, these chromosomes would carry nearly identical genes in spite of their independent origins; the population is genetically homogeneous. If, in addition, heterozygous combinations were made of different pairs of these chromosomes and recombination chromosomes were obtained by crossing over, the recombinants within the array recovered from each combination and the arrays recovered from different combinations would be uniform.

Within a second population, using the same assumptions, one can postulate the same genetic situation, i.e., genetic homogeneity, without postulating that the alternative alleles found within the second population are identical with those of the first. The alleles found in the second population may be represented as *A′–a′*, *B′–b′*, etc. Recombination between pairs of chromosomes, one of which comes from one population and the other from the second, can result in a variable array of crossover products. For instance, using the above models, an inter-population heterozygous combination would have the genetic constitution *AB/A′B′*; four chromosomal types—*AB*, *A′B*, *AB′*, *A′B′*—can be recovered from this combination. These four types, when homozygous, may produce entirely different phenotypes. Nevertheless, because of the similarity of chromosomes within each population practically all of the inter-population heterozygotes would be *AB/A′B′*. Every combination, then, would have the capacity to form the same variable array of crossovers as any other. Therefore, in both the intra- and inter-population chromosomal combinations arrays of recombination products would be expected to be similar from *combination* to *combination*.

An extreme alternative genetic situation can be imagined in which individuals heterozygous for certain alleles possess adaptive values exceeding those possessed by any homozygous combination and, further, in which the adaptive value of any particular combination of alleles depends upon its interactions

with the combinations at all other loci. The result of such a system would be (1) the maintenance in the population of a variety of alleles at each locus and a consequent decrease in the frequency of homozygosis for any one allele (similar to the self-sterility genes in certain plants) and (2) the integration of these alleles by selection into the coadapted complex producing the highest average adaptive value for all likely combinations. If samples of chromosomes were taken from two populations of this kind it might be expected that the arrays

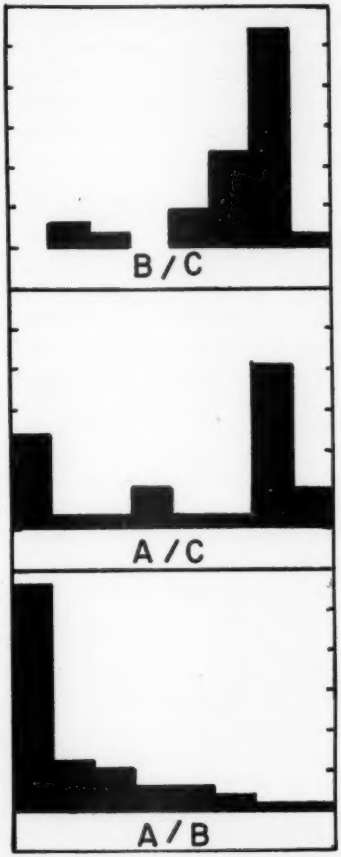


FIGURE 1.—Viability of individuals homozygous for recombinant second chromosomes recovered from female *Drosophila pseudoobscura* heterozygous for the three possible combinations of chromosomes A, B and C. Horizontal axis: Viability expressed in percent of wild flies in test cultures—0–5, 5–10, 10–15, 15–20, 20–25, 25–30, 30–35 and 35–40. Vertical axis: Percent of sample; each division equals 10%. (Data from tables 1, 3 and 5, DOBZHANSKY 1946.)

of recombinants recovered from the various paired combinations, either intra- or inter-population, need not be similar. Furthermore, since the alleles present in one population are selected in relation to the alleles existing at other loci in the same population, it might also be expected that interbreeding of members of these populations would give rise to unselected combinations of alleles and would often produce ill-adapted recombination products.

The two schemes outlined above are not mutually exclusive; it is conceivable that alleles at some loci behave in one way and those at other loci behave in a different manner. Nor is there any way of estimating the number or proportion of loci behaving in either way. Unpredictable interactions among the recessives postulated by the first scheme would easily be confused with the expectations of the second. However, " unpredictable interactions " implies that a in a certain genetic background behaves as a' or a''; this, for our purposes, is the equivalent of a multiple allelic series.

AN EARLY EXPERIMENT

An analysis of many crossover chromosomes from a series of different chromosomal combinations entails a great deal of labor. It was encouraging, therefore, that DOBZHANSKY (1946) had done a relevant experiment using chromosomes from natural populations of *D. pseudoobscura;* with his permission, some of his data are reproduced in figure 1. Three chromosomes were taken from two populations inhabiting localities separated by about 15 miles. Chromosome A was lethal when homozygous at 25°C, the temperature of the experiment; chromosomes B and C when homozygous gave good viabilities and were quite similar in their effects on both viability and rate of development. Homozygous crossover recombinants from B/C females were quite normal in viability except for a small number of subvitals. Recombinants from females heterozygous for A and C had a great range of viabilities; quite a number were lethal (some of these were undoubtedly non-crossover A's); some were semilethal; and a large portion were normal in viability. The recombinants of A/B heterozygotes, on the other hand, were nearly all lethal or semilethal. Chromosomes B and C that appeared so similar on the basis of their behaviors when homozygous and, further, by the uniformity of their various crossover products, are clearly different. There is scarcely a recombinant of A with B that possesses a fair viability when homozygous. Three chromosomes scarcely present a " proof " but it is noteworthy that A and C were from the same locality while B had a different geographical origin. The higher mean viability of the intra-population homozygous recombinants could be interpreted as a manifestation of " coadaptation."

MATERIAL AND METHODS

The chromosomes used in this study were obtained from two experimental populations of *D. melanogaster* designated as #1 and #3. These two populations were started about 60 generations before the present analysis. The original parents of the populations carried nearly identical proportions of several

lethal-free second chromosomes obtained from a mass culture of Ore-R flies. The parental flies of population 1 were exposed to a massive dose of X-radiation (7000 r for males, 1000 r for females). Detailed accounts of these populations have been given by WALLACE and KING (1951) and WALLACE (1951). Experimental populations were chosen in preference to natural populations because their histories were well known and, consequently, effects similar to those reported by DOBZHANSKY, if found, would have added significance.

Five second chromosomes, viable when homozygous, were chosen from chromosomes isolated and tested during routine genetic analyses of each population. The chromosomes chosen from population #1 were designated *1, 2, 3, 4* and *5;* those from population #3, *a, b, c, d* and *e*. Except that *4, 5, d* and *e* were chosen for their poor viabilities when homozygous and the others for their "normal" viabilities, no particular choice was exercised in the selection. Lethals were avoided in an effort to keep the proposed experimental design orthogonal. Cytological examination of salivary gland chromosomes revealed no detectable aberrations among these ten chromosomes.

After the ten chromosomes had been selected, *Cy L/+* flies from the balanced stocks were intercrossed (see fig. 2) to produce the ten homozygous classes of wild type flies (*1/1, 2/2, 3/3, 4/4, 5/5, a/a, b/b, c/c, d/d* and *e/e*), the 20 classes of intra-population heterozygotes (*1/2, 1/3, 1/4, 1/5, 2/3, 2/4, 2/5, 3/4, 3/5, 4/5, a/b, a/c, a/d, a/e, b/c, b/d, b/e, c/d, c/e* and *d/e*), and the 25 classes of inter-population heterozygotes (*1/a, 1/b, 1/c, 1/d, 1/e, 2/a, 2/b, 2/c, 2/d, 2/e, 3/a, 3/b, 3/c, 3/d, 3/e, 4/a, 4/b, 4/c, 4/d, 4/e, 5/a, 5/b, 5/c, 5/d* and *5/e*). Only a single generation elapsed between the time the ten chromosomes were chosen and the matings outlined above; this generation was needed to increase the numbers of *Cy L/+* males and females available. The origin of three of these combinations as well as the subsequent mating procedure is represented in figure 2.

Four wild type virgin females (P_1) of each of the 55 possible combinations were crossed singly to *Cy L/Pm* males; the four females served as replicas of each genotypic combination. In cases of obviously poor fertility, additional P_1 females of the proper genotypes were mated in an effort to obtain more offspring; the homozygous females of population 3 had obviously poorer fertility than those of population 1. Wherever possible, 30 *Cy L/+* F_1 males were obtained from each of the P_1 females. Each of these males carried a wild type chromosome that, unless it happened to be a non-crossover, was a composite of the two chromosomes carried by the P_1 female. We can assume (RHOADES 1931) that about 30% of the chromosomes recovered from the P_1 females were non-crossovers; about 70% were crossovers of one sort or another.

The *Cy L/+* F_1 males were mated singly to *Cy L/Pm* females in vials. Two to four *Cy L/+* F_2 females (virgin) and males were collected from each vial and again mated in vials. These F_3 cultures were expected to produce *Cy L/+* and +/+ flies in a 2:1 ratio. The wild flies in these vials were ***homozygous*** for whatever combination of second chromosome genes crossing over happened to produce. The homozygous P_1 females presumably gave rise only to replicas of

the original chromosomes; intra-population heterozygous P_1 females gave rise to chromosomes that could have been produced as easily in the populations themselves; and the inter-population heterozygous P_1 females produced combinations that could arise only through this deliberately arranged hybridization.

The homozygous recombinants of the F_3 cultures were studied for: (1) the frequency of lethals, (2) the viability of non-lethals, and (3) the number of

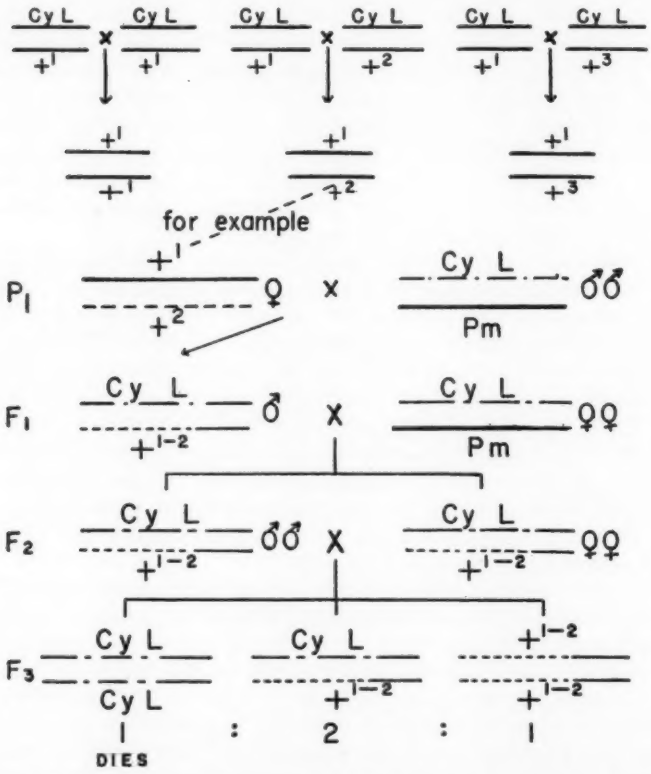


FIGURE 2.—Mating scheme for recovering individuals homozygous for crossovers between pairs of second chromosomes in *D. melanogaster*. The formation of combinations 1/1, 1/2 and 1/3 is indicated; a detailed account of only 1/2 is given as an example.

abdominal bristles on non-lethal females. In addition, since *a*/*a* females proved to be sterile, tests for sterility among females homozygous for chromosomes derived from combinations involving chromosome *a* were made as a fourth analysis.

The details of the experimental procedures of the different analyses will be described below. It is sufficient at present to note that all tests were made at 25°C. The F_2 and F_3 vials contained cornmeal-agar-molasses medium enriched

with brewer's yeast. The F_3 cultures were examined on the 14th day after the matings. It was impossible to randomize the matings of P_1 females but the following schedule was used: two P_1 females of different homozygous combinations of each population, four P_1 females of different intra-population heterozygous combinations of each population, and ten P_1 females of different inter-population combinations were mated each day. The ages of P_1 females of different groups were comparable; within any group the ages at mating varied by four days. The F_3 vials were mated within an 11-day period in the early part of March 1952 and the final examinations were made over an 11-day interval at the end of the same month.

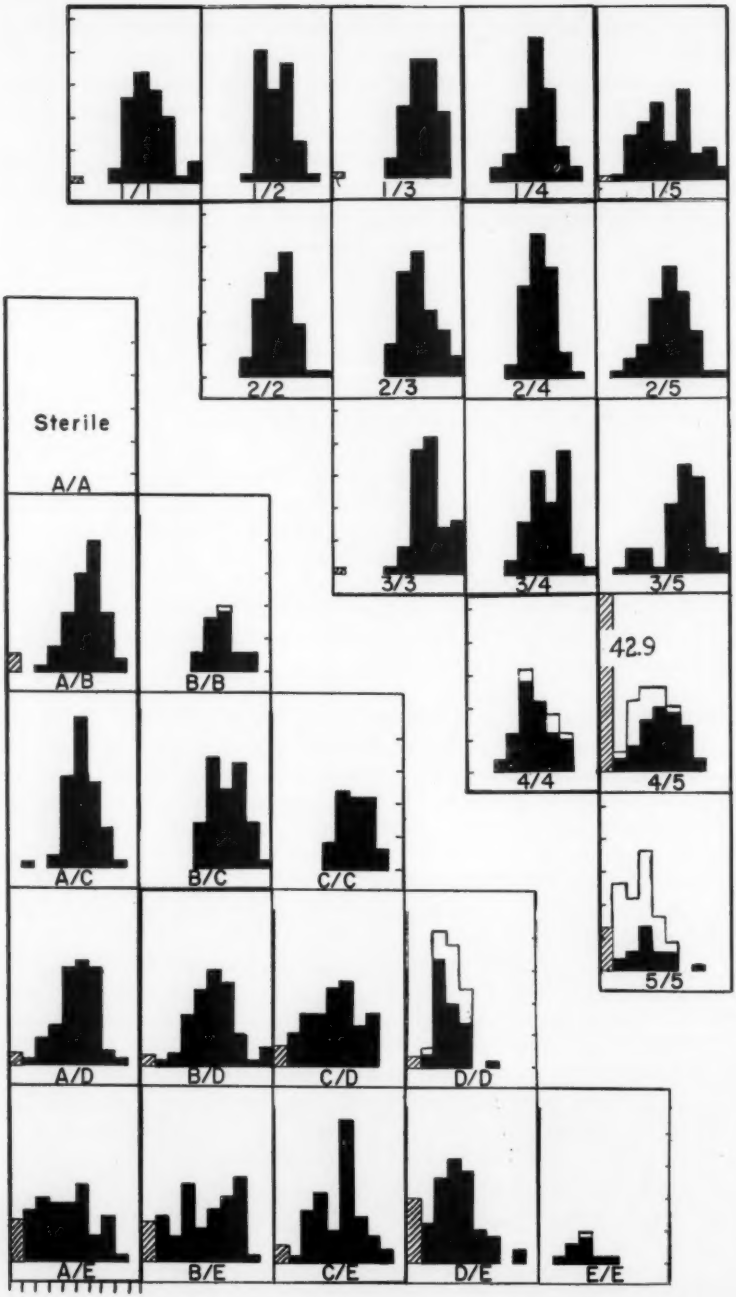
DESCRIPTIVE ANALYSIS

Lethals and nonlethals

The lethal and viability studies may well be considered together. For convenience of statistical analysis and for other practical considerations, it was decided in advance that the measurement of viability would be the number of wild flies in a sample of fifty taken at random from each of the F_3 cultures. Preliminary trials indicated that Curly-Lobe and wild flies are distributed randomly when mixtures of flies are placed on a counting plate and aligned in single file. The constant sample size simplified the data in that only the number of wild type flies was recorded. Orthogonality was destroyed by the sterility of a/a females and by the reduced fertility of some other types of females; consequently, attempts to maintain identical numbers of flies tested for lethals were abandoned. No more than 60 vials were counted per combination for viability studies, however.

The experimental procedure consisted of placing etherized flies from each F_3 vial onto a counting plate. Without recourse to the microscope, an estimated fifty of them were aligned in single file. Then, with the microscope, all of the flies were examined for the presence of wild type individuals. The absence of wild type flies in a vial of more than 20 individuals constituted a lethal. (Certainly no more than 2% of the vials had fewer than 50 flies; the vast majority had between 75 and 100.) If there were no wild flies in a vial of less than 20 flies, a confirmation (F_4) cross was made; about six of these confirmations were required and they all proved to be lethal.

If there were one or more flies in a vial, that chromosome was classified as non-lethal. Fifty flies were then counted in the pre-arranged row and the number of wild flies among the fifty was recorded. Occasionally, especially in the case of chromosomes of very poor viability, there were only one or two wild flies in the entire vial; if these were not included among the 50, the chromosome was classified as a non-lethal but with zero wild type flies. It should be noted that vials which produced fewer than fifty flies could not be classified as non-lethal with no wild flies; they were either lethal or non-lethal with wild flies present. The latter vials are included in the table and figure (outlined in fig. 3) only if there were fewer than 60 non-lethal cultures with at least fifty flies.


1/1
1/2
1/3
1/4
1/5
2/2
2/3
2/4
2/5
Sterile
A/A
3/3
3/4
3/5
A/B
B/B
42.9
4/4
4/5
A/C
B/C
C/C
5/5
A/D
B/D
C/D
D/D
A/E
B/E
C/E
D/E
E/E

Lethals were not subcultured for confirmation because this study required only internal consistency. In an earlier experiment (WALLACE 1950) lethals were screened in vials and were then re-tested in regular half-pint cultures. In that experiment 85% of the suspected lethals were confirmed, and it seems likely that a similar figure would apply in the present case.

The results of the lethal tests are shown in the cross-hatched columns of figure 3, and in table 1 where the 55 combinations of the ten chromosomes are listed at the left. The figures in fractional form show the number of lethal chromosomes and the total number of chromosomes tested for each of the different P_1 females (represented at the top of the table by Roman numerals). In six cases (*1/4* I, *a/c* I, *b/c* I, *1/a* III, *2/b* II, and *4/b* III) one of the females gave strikingly more lethals than did the others of the same genotype; these females are not included in the totals at the right. The close approximation to a 1 : 1 ratio shown by these aberrant individuals indicates that they carried lethal mutations that had occurred before the P_1 generation; the six lethals in 26 tests of *b/c* I may have come from an ovary mosaic for lethal-bearing tissue.

It is evident from the table that the frequency of lethals recovered from females of different genotypes is not uniform. It varies in the present case from a high of 43%, through 10%, 9%, 8%, and so on down to many cases of 0%. The calculated frequencies of lethals are lower than the frequencies with which lethals arise by recombination since the calculation is based on the total chromosomes tested; as high as 30% of these tested chromosomes may be non-crossover, non-lethal replicas of the original ones.

An inspection of figure 3 reveals a number of chromosomal combinations that gave rise to many recombinants of extremely poor viability—including lethals as the lower limit of the analyzable distribution. In a number of instances the lethal recombinants are a discrete class. Chromosomes *1, 2, 3, a, b* and *c* were chosen for their good viability when homozygous; nevertheless, 2–3% lethals are found among the recombinants of *a* and *b, 1* and *b, 2* and *b, 3* and *b,* and *1* and *c*. As we will see later, chromosomes *3* and *c* had the two highest viabilities when homozygous and yet 5% of their recombinants were homozygous lethals!

The highest frequency of lethals within any combination arose through crossing over between chromosomes *4* and *5*. The similarity of the data obtained from all four P_1 females eliminates the possibility that a previous lethal mutation was responsible; this frequency of lethals is characteristic of the

FIGURE 3.—The array of viabilities of individuals homozygous for recombinant chromosomes recovered (1) from females of *D. melanogaster* homozygous for 5 different chromosomes isolated from each of two populations or (2) from females heterozygous for pairs of the chromosomes obtained from the same population. Horizontal axis: The divisions indicated beneath a/e represent, from left to right, lethals (cross hatched) and viability of non-lethals (black or white) based on the number of wild flies in samples of fifty—0–2, 3–5, 6–8, 9–11, 12–14, 15–17, 18–20, 21–23, 24 or more. Vertical axis: The divisions indicate per cent lethal or number of non-lethal chromosomes; each division is 5% or 5.

TABLE 1

The frequency of lethals arising through recombination. 1/1... 1/2... a/a... a/b... 1/a... 5/e represent the 55 different combinations of 10 chromosomes, 1... 5, a... e. Roman numerals represent different females carrying the same combination of chromosomes. The numbers in fractional form are the numbers of lethals found among the total chromosomes tested. Asterisks mark females presumably heterozygous for lethals of pre-recombination origin.

Genotype	I	II	III	IV	V	VI	VII	VIII	Total	%
1/1	0/30	1/27	0/28	0/28	....	....	....	....	1/113	0.9
2/2	0/30	0/22	0/17	0/28	0/17	....	....*	....	0/114	0
3/3	0/26	0/28	0/27	1/27	....	....	....	....	1/108	0.9
4/4	0/2	0/3	0/5	0/14	0/13	0/3	0/6	0/4	0/50	0
5/5	0/26	0/1	2/5	1/4	1/7	0/8	0/11	....	4/62	6.5
1/2	0/30	0/28	0/29	0/25	....	....	....	....	0/112	0
1/3	1/29	0/30	0/31	0/29	....	....	....	....	1/119	0.8
1/4	13/29*	0/29	0/25	0/28	....	....	....	....	0/82	0
1/5	0/29	1/28	0/27	0/26	....	....	....	....	1/110	0.9
2/3	0/27	0/26	0/27	0/29	....	....	....	....	0/109	0
2/4	0/29	0/27	0/26	0/30	....	....	....	....	0/112	0
2/5	0/25	0/29	0/29	0/27	....	....	....	....	0/110	0
3/4	0/27	0/26	0/29	0/29	....	....	....	....	0/111	0
3/5	0/27	0/30	0/22	0/21	....	....	....	....	0/100	0
4/5	10/29	10/27	15/28	13/28	....	....	....	....	48/112	42.9
a/a	Females are sterile									
b/b	0/3	0/3	0/21	....	....	....	....	....	0/27	0
c/c	0/26	0/2	0/4	0/9	....	....	....	....	0/41	0
d/d	0/6	0/3	0/6	0/5	0/7	1/28	....	....	1/55	1.8
e/e	0/11	....	....	....	....	....	....	....	0/11	0
a/b	1/26	1/32	0/13	0/10	1/30	....	....	....	3/111	2.7
a/c	12/29*	0/27	0/27	0/27	....	....	....	....	0/81	0
a/d	0/28	0/29	1/27	1/21	....	....	....	....	2/105	1.9
a/e	1/29	0/25	4/27	2/30	....	....	....	....	7/111	6.3
b/c	6/26*	0/26	0/26	0/24	....	....	....	....	0/76	0
b/d	0/28	0/24	2/26	0/27	....	....	....	....	2/105	1.9
b/e	3/30	0/26	1/22	2/20	....	....	....	....	6/98	6.1
c/d	1/25	0/21	1/29	1/25	....	....	....	....	3/100	3.0
c/e	2/26	0/30	1/27	0/19	....	....	....	....	3/102	2.9
d/e	0/26	0/5	3/28	5/26	3/28	....	....	....	11/113	9.7
1/a	0/30	1/30	16/30*	0/27	....	....	....	....	1/87	1.1
1/b	1/29	2/28	0/30	0/29	....	....	....	....	3/116	2.6
1/c	0/28	1/29	0/26	2/30	....	....	....	....	3/113	2.7
1/d	0/29	0/29	0/30	0/28	....	....	....	....	0/116	0
1/e	1/28	1/29	1/27	2/28	....	....	....	....	5/112	4.5
2/a	0/32	0/28	0/28	0/30	....	....	....	....	0/118	0
2/b	0/27	10/26*	2/26	0/33	....	....	....	....	2/86	2.3
2/c	0/29	0/28	0/34	0/29	....	....	....	....	0/120	0
2/d	0/30	0/30	0/21	0/28	....	....	....	....	0/109	0
2/e	0/27	0/28	0/24	0/29	....	....	....	....	0/108	0
3/a	0/29	0/28	0/37	0/28	....	....	....	....	0/122	0
3/b	0/29	1/29	0/23	1/28	....	....	....	....	2/109	1.8
3/c	2/32	2/29	2/28	0/27	....	....	....	....	6/116	5.2
3/d	1/28	0/31	0/31	0/27	....	....	....	....	1/117	0.9
3/e	0/30	2/30	0/30	1/28	....	....	....	....	3/118	2.5
4/a	0/30	0/29	0/16	0/41	....	....	....	....	0/116	0
4/b	0/29	1/22	11/27*	0/15	....	....	....	....	1/66	1.5
4/c	0/29	0/26	0/19	0/27	....	....	....	....	0/101	0
4/d	0/24	1/24	0/31	1/34	....	....	....	....	2/113	1.8
4/e	0/28	6/29	3/27	1/28	....	....	....	....	10/112	8.9
5/a	0/27	0/29	3/50	1/30	....	....	....	....	4/136	2.9
5/b	0/23	4/21	3/39	2/33	....	....	....	....	9/116	7.8
5/c	1/30	1/27	0/29	0/24	....	....	....	....	2/110	1.8
5/d	0/27	1/29	0/27	0/24	....	....	....	....	1/107	0.9
5/e	0/16	0/10	0/25	0/31	1/25	....	....	....	1/107	0.9

combination. More will be said of this combination later but we might note one fact here: these chromosomes, *4* and *5*, were chosen because of their poor viability. The lethals formed by recombination, however, are not the result merely of recombining two genes, one from *4* and one from *5*, into a lethal complex; such a simple recombination would produce at most only 25% lethals and would probably result equally often in non-lethal recombinations with correspondingly higher viabilities.

The importance of these data lies in their demonstration of the variety of phenotypic effects which a gene—a physical unit of the chromosome—can produce when found in various combinations and the high frequency with which the necessarily different combinations arise. The only circumstances under which a phenotype need show the classical Mendelian inheritance pattern are (1) if the chromosomal material uniquely involved in the expression of a particular phenotype manifests itself in nearly all genetic backgrounds or (2) if the genetic background is so uniform that the only effective segregation is for the locus in question. In ordinary genetic studies the genes are generally of the first type, or stocks are established by selection (perhaps unwittingly in the process of isolating homozygous stocks) in which the gene studied is the only effectively segregating unit. We now see that even young populations of Drosophila possess sufficient genetic diversity to alter, through new combinations, the phenotypic effects of genes at various loci.

TABLE 2

*The mean viabilities of non-lethal recombinant chromosomes recovered from different combinations (1/1, 1/2... 5/e) of ten chromosomes (1, 2,... e). The viability is expressed in terms of the average number of wild flies observed in samples of 50.**

1/1	14.58	*5/5*	6.49	*d/e*	8.18	*3/c*	16.23
1/2	13.53	*a/a*	....	*e/e*	6.51	*3/d*	11.18
1/3	16.98	*a/b*	14.33	*1/a*	13.75	*3/e*	13.30
1/4	13.32	*a/c*	13.28	*1/b*	14.98	*4/a*	13.48
1/5	12.82	*a/d*	15.07	*1/c*	14.63	*4/b*	15.12
2/2	14.08	*a/e*	9.48	*1/d*	12.07	*4/c*	13.82
2/3	16.47	*b/b*	15.54	*1/e*	9.95	*4/d*	10.55
2/4	13.55	*b/c*	16.15	*2/a*	14.65	*4/e*	6.90
2/5	12.95	*b/d*	12.87	*2/b*	14.32	*5/a*	11.50
3/3	18.72	*b/e*	11.43	*2/c*	14.93	*5/b*	14.02
3/4	15.53	*c/c*	15.71	*2/d*	11.45	*5/c*	13.63
3/5	15.33	*c/d*	10.52	*2/e*	9.53	*5/d*	9.33
4/4	12.26	*c/e*	11.37	*3/a*	16.55	*5/e*	7.65
4/5	9.55	*d/d*	6.54	*3/b*	15.48		

Several copies of the raw data upon which this table and figure 2 are based have been deposited with the editorial office of Genetics.

In addition to the lethals discussed above and the viability of non-lethals that follows, there was one outstanding case of a visible mutation dependent upon recombination. Among the non-lethal recombinants of *d* and *e* (about 10 instances or, roughly, about 10%) an eye mutant appeared—bulging eye, darker than wild type, "pebbly" facets, and devoid of the normal nap of fine bristles—that was clearly distinct from the normal eye. This aberrant pheno-

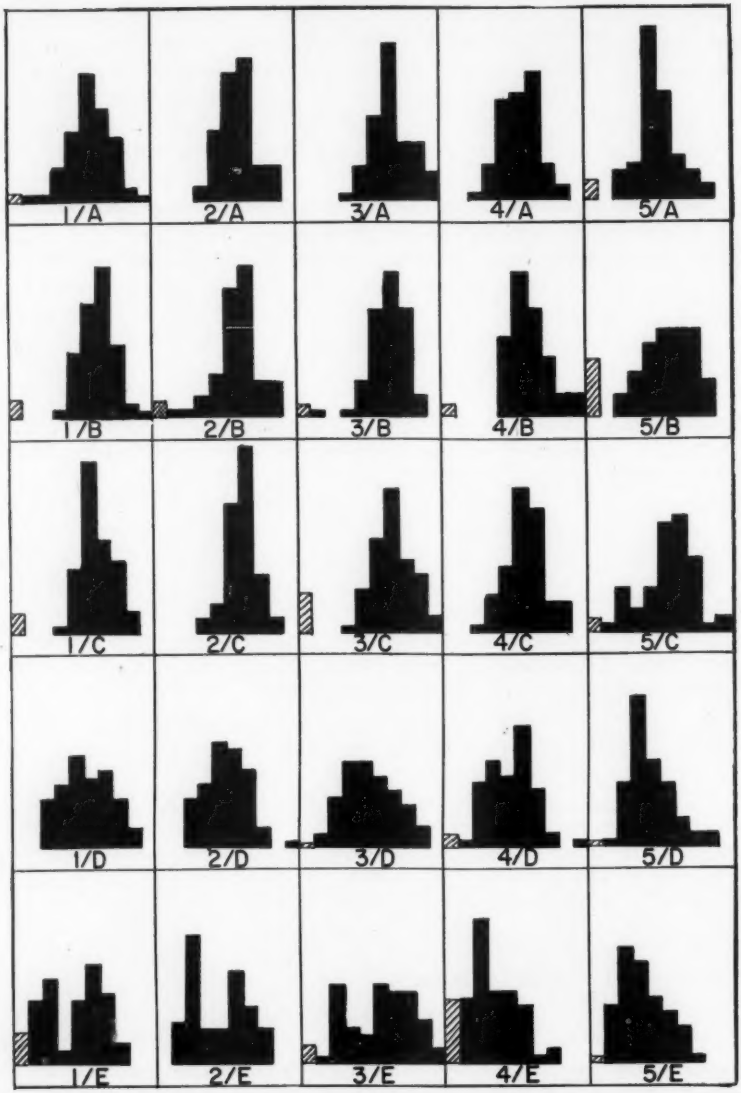


FIGURE 4.—The array of viabilities of individuals homozygous for recombinant chromosomes recovered from females heterozygous for the 25 possible combinations of the five chromosomes from each of two populations. Horizontal and vertical axes as in figure 3.

type was not present in *d/d* or *e/e* individuals or in any of the recombinants of these chromosomes with any of the others.

The data of the viability of non-lethal homozygous recombinants are given in table 2 and figures 3 and 4. The table gives the mean numbers of wild flies found in the samples of 50. (The data for each vial are too extensive for publication; mimeographed copies can be obtained from the authors or from the editor of GENETICS.) In the figure, the determinations based on 50 flies are shown in black; the determinations based on counts of fewer than 50 flies are white.

The relative viabilities of individuals homozygous for the original chromosomes can be calculated from the data on recombinants derived from homozygous P_1 females. The non-lethal chromosomes obtained from homozygous P_1 females of population #1 had the following mean viabilities: (*1*) 14.6, (*2*) 14.1, (*3*) 18.7, (*4*) 12.3, and (*5*) 6.5. The corresponding data for chromosomes from population #3 are: (*a*) unknown because of the female sterility, estimated later as about 12.0, (*b*) 15.5, (*c*) 15.7, (*d*) 6.5, and (*e*) 6.5. The number of flies expected in these samples on the basis of the theoretical 2:1 ratio is 16.7.

A preliminary examination of the data can be made without recourse to statistics; a more refined analysis will be presented in the following section. One can select from the figure pairs of chromosomes that appear to be similar (either because they give approximately the same viability when homozygous or, better, because the crossover products of the heterozygous combination of the two have highly uniform viabilities) and then compare the recombinants from these chromosomes in other combinations. For instance, chromosomes *d* and *e* gave almost identical viabilities when homozygous; moreover, they came from the same population. One might suspect that they were basically very similar. However, the patterns of the viabilities of their recombinants with any of the other eight chromosomes quickly dispels this impression.

A number of heterozygous combinations gave rise to recombinants possessing remarkably uniform viabilities—*1/2, 2/4, a/c, 2/c* and (if the lethal is disregarded) *1/3,* for example. It seems reasonable to assume that if all crossover strands recovered from a given pair of chromosomes are similar, the two original chromosomes must also have been similar. If, for example, an array of chromosomes derived from a female of unknown genotype were all *w m f* one would suspect that the female was homozygous for these three genes.

To detect possible differences between chromosomes suspected of being similar, the recombinants of the two were examined when each was in combination with a third. Chromosomes *1* and *2* gave recombinants that possessed extremely uniform viabilities; the recombinants of *1* and *5,* however, formed a bimodal curve that included one lethal while *2* and *5* produced a unimodal distribution. Furthermore, *1* and *a* produced several recombinants of very poor viability (one lethal among them) while *2* and *a* did not; *1* and *c* gave rise to 3% lethals while *2* and *c* produced none; *1* and *e* produced a bimodal distribution that included 5% lethals while *2* and *e* produced a bimodal distribution with lethals conspicuously absent.

Another pair of chromosomes that appeared similar on the basis of their recombinations was *2* and *4*. Compare, however, the distributions obtained from *2/2* and *4/4* homozygous P_1 females. Compare also *2/5* and *4/5*, *2/b* and *4/b* (here *2*, not *4*, gave rise to the recombinants with poor viability), *2/d* and *4/d*, and *2/e* and *4/e*. (The symbols *2/3*, *1/a*, *c/e*, etc., used in this paper refer to the chromosomal combinations of the P_1 females; the tested chromosomes, as has been repeatedly emphasized, are homozygous.)

The other pairs can be analyzed similarly. In the case of *a* and *c* examine their recombinants with *b*, *d*, *e* (very striking), *1*, *3* (lethals formed by *3/c*) and *5*. For *2* and *c* one can check their behavior with *1* (lethals with *c*), *3* (lethals, again), *5*, *b*, *d* and *e*. Finally, in the case of *1* and *3*, there is the lethal produced by *1/3* (mutation? recombination?) and the behavior of the two chromosomes with *5* and *e*. In each case considered, apparent similarities prove to be questionable when the examination is pressed further. Homozygosis for chromosomes carrying various combinations of genes frequently produces results unexpected on the basis of the behavior of the same genes in other combinations; the epistatic properties of corresponding chromosomal regions of the different tested chromosomes are not identical. Genetic differences do exist between materials found in different chromosomes and different homozygous combinations of these materials result in interactions specific for different combinations.

Bristles

The bristle analysis was made to obtain information concerning a system other than viability known to be controlled in part by the genotype (MATHER 1949, and earlier papers). Since there is no obvious relation between numbers of abdominal bristles and survival value, it could be not predicted whether the results of this study would merely repeat the results of the viability analysis. It was felt, though, that comparable studies of two distinct phenotypic traits within the same material would greatly increase the value of the entire experiment.

Unfortunately, limitations of time curtailed this analysis severely. The prospect of counting was not the only deterrent; the interruptions necessitated by sorting out females, preserving them in vials of alcohol, recording their preservation, and checking the records to see if females were required from the next vial, minor as they now seem, were too much to tolerate when hundreds of vials were being counted daily. The analyses were made on wild females from non-lethal F_3 cultures. Four females from one vial from each of the original P_1 females were preserved in alcohol and, within a day or so, the number of bristles on the 6th abdominal sclerite was determined under 40× magnification. All of these counts were made by one person (B. W.) in order to avoid personal differences such as has been reported by MATHER and HARRISON (1949).

The data on bristle numbers are given in table 3 and figures 5 and 6. The results are less striking than those of the lethals and non-lethals. All of the

TABLE 3

Numbers of bristles on the 6th abdominal sternite of females homozygous for recombinant chromosomes. The chromosomes (1... e), the combinations (1/1... 5/e), and the different females (I, II, etc.) are the same as those of table 1.

	I		II		III		IV	
1/1	18	20	19	21	20	20	18	20
	19	22	19	20	20	20	22	19
1/2	20	21	23	22	19	22	20	19
	22	20	18	23	19	22	24	22
1/3	20	20	19	16	17	18	19	17
	20	21	18	19	18	21	20	17
1/4	20	19	20	23	20	18	16	17
	18	18	21	20	20	19	17	17
1/5	20	22	19	17	17	22	17	21
	19	20	21	18	19	23	19	22
2/2	19	20	23	20	21	21	18	22
	20	22	18	18	24	19	20	21
2/3	21	22	19	17	17	17	20	17
	19	23	18	20	18	20	17	18
2/4	19	22	21	21	19	20	20	21
	22	19	23	21	22	17	23	20
2/5	19	26	24	21	21	20	20	19
	22	20	21	19	21	18	20	23
3/3	19	18	17	19	19	20	21	15
	20	19	17	19	20	18	17	17
3/4	23	20	16	18	17	16	18	16
	18	21	20	17	17	17	15	19
3/5	18	17	17	15	18	19	18	22
	14	15	17	18	18	17	16	18
4/4	20	21	17	20	23	20	....	....
	21	25	20	19	22	19	....	....
4/5	20	21	18	20	....	....	22	16
	19	20	22	21	....	....	18	16
5/5	....	....	22	22	17	18	19	19
	....	....	17	20	21	19	20	21
a/a			**Females are sterile**					
a/b	18	18	20	21	19	17	16	19
	17	21	20	17	19	18	20	18
a/c	....	....	17	18	18	19	18	20
	....	....	19	22	18	20	19	19
a/d	18	17	18	16	18	18	19	20
	17	19	16	17	20	19	18	21
a/e	19	19	21	18	25	18	14	16
	20	16	20	14	16	16	20	17
b/b	23	22	20	17	21	22	....	....
	21	24	18	18	17	16	....	....
b/c	19	21	18	20	22	19	20	20
	20	18	18	19	21	21	22	17

TABLE 3 (*continued*)

	I		II		III		IV	
b/d	19	21	19	20	19	19	19	17
	17	19	18	21	17	18	16	22
b/e	17	20	15	19	19	19	21	22
	16	18	16	21	18	16	20	18
c/c	21	20	18	18	....	....	....	....
	19	19	20	22	....	....	....	....
c/d	20	23	22	19	20	18	17	17
	23	19	20	21	17	18	20	22
c/e	19	19	16	20	19	21	18	20
	19	19	18	23	21	21	20	22
d/d	22	19	22	18	....	....	18	16
	17	18	25	17	....	....	16	17
d/e	16	21	17	17	19	21	17	19
	16	20	19	20	20	20	17	22
e/e	Too inviable to get the required four females per vial.							
1/a	23	23	15	17	17	17	....	....
	22	18	15	15	17	19	....	....
1/b	19	19	17	18	20	22	18	22
	16	18	17	18	21	22	20	21
1/c	18	20	20	21	22	22	19	20
	18	19	22	19	21	20	20	22
1/d	19	18	20	21	18	22	22	23
	17	19	18	17	20	25	21	20
1/e	19	23	23	19	22	19	20	19
	20	19	15	19	20	20	17	22
2/a	16	15	14	19	17	20	18	21
	17	18	17	18	21	20	21	17
2/b	21	25	21	22	19	19	22	22
	24	19	20	22	20	15	19	20
2/c	17	16	23	22	17	19	19	20
	19	18	22	22	17	20	18	19
2/d	19	24	21	19	21	18	21	20
	23	21	18	16	16	18	19	18
2/e	19	22	22	22	22	21	22	16
	23	20	21	23	23	19	19	25
3/a	18	17	16	18	19	18	16	12
	17	16	16	16	18	17	16	12
3/b	18	18	20	17	20	18	21	15
	17	18	17	17	20	18	19	20
3/c	17	19	19	19	19	20	17	17
	17	18	19	19	17	18	17	21
3/d	15	19	16	17	19	15	16	21
	19	19	20	17	19	21	18	23
3/e	17	18	19	16	17	17	20	19
	19	20	19	18	24	20	17	21
4/a	19	19	17	18	21	22	17	17
	18	18	21	21	20	21	22	20

TABLE 3 (*continued*)

	I		II		III		IV	
4/b	20	21	18	18	19	19	20	21
	20	21	20	20	21	22	25	22
4/c	20	19	19	18	20	20	19	22
	18	21	19	16	22	20	22	18
4/d	19	20	19	20	20	17	18	18
	17	19	20	19	20	18	19	20
4/e	22	20	17	21	19	18	18	23
	21	18	19	17	20	18	22	20
5/a	17	15	15	19	17	18	20	15
	15	18	16	17	18	19	16	21
5/b	....	....	22	15	20	18	18	20
	....	....	21	19	22	21	18	17
5/c	18	18	22	19	22	17	16	19
	17	21	21	18	19	20	20	15
5/d	21	19	20	16	22	21	22	20
	20	16	20	21	22	22	20	20
5/e	19	22	18	17	18	20	16	21
	18	21	24	16	19	20	17	20

***Not included in the above counts are two females (1/1 IV and 3/c II) in which the abdominal sclerites were incompletely formed and were lacking one side or the other.**

flies observed had between 12 and 16 bristles; although no vial had a range this large, a number of combinations (*d/d, 1/a, 2/b, 3/d* and *3/e*) had ranges nearly as great. A casual examination of these figures fails to reveal any very striking deviations similar to those noted in the two previous studies. A more formal demonstration of differences will be made later.

Sterility

Advantage was taken of the unexpected female sterility of *a/a* homozygotes to test still another trait. In order to find out how this sterility behaved in recombination, six non-lethal cultures (F_3) were chosen from each of the four P_1 females of all combinations involving chromosome *a* and the wild females in these cultures were tested for sterility. From seven to twelve non-virgin females and an equal number of their Curly-Lobe brothers were placed in a fresh vial. After a week these vials were examined for signs of larvae and pupae; those that were devoid of such signs (as were the original cultures of *a/a* females) were scored as sterile.

If the cause of the sterility behaved as a single gene, one half of the recombinations should prove fertile and the other half sterile. If, however, the sterility resulted from a more complex interaction, so simple a ratio would probably not occur. The data (table 4) fit the single gene hypothesis very well. In 211 tests, 103 proved to be sterile and 108 fertile. A Brandt-Snedecor test for homogeneity of the distribution of sterile tests among the different combinations fails to reveal any heterogeneity; the Chi-square of 11.7 with 8 degrees

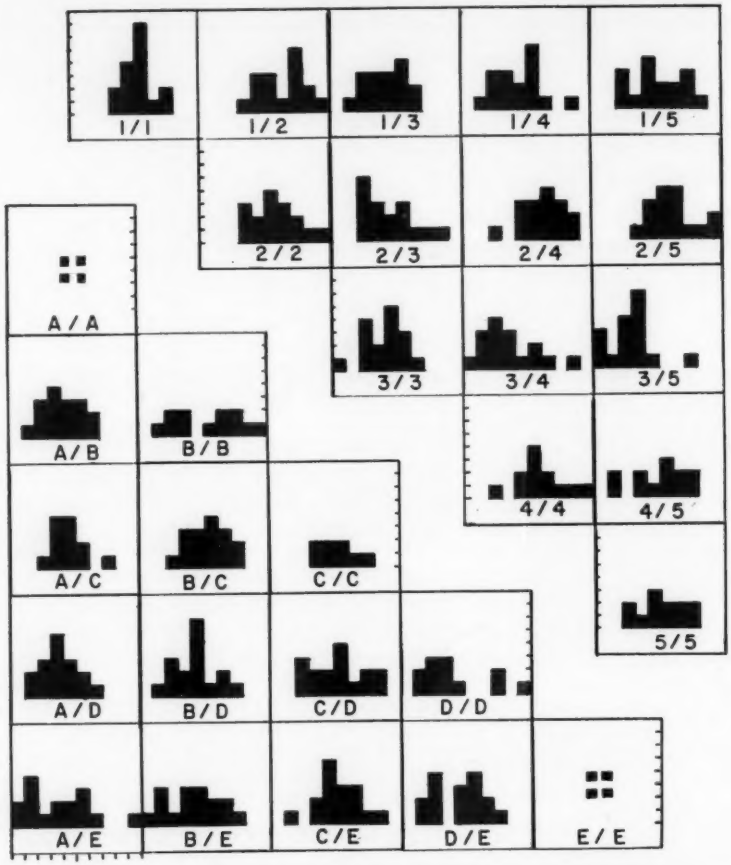


FIGURE 5.—Number of bristles on the 6th abdominal sternite of females homozygous for recombinant chromosomes recovered from females homozygous for each of 10 chromosomes (1 2, ... e) or from females heterozygous for combinations of chromosomes from the same population. Vertical axis: Numbers of females; each division is a single female. Horizontal axis: Number of bristles; 12–15, 16, 17, 18, 19, 20, 21, 22, 23, 24–26.

of freedom has a probability of .15. Consequently, it appears that none of the chromosomes chosen from either population reacts in unexpected fashion with the sterility factor of chromosome *a*.

STATISTICAL ANALYSIS

The results of the statistical analysis of the data are presented in this separate section for a number of reasons. First, none of the authors is an accom-

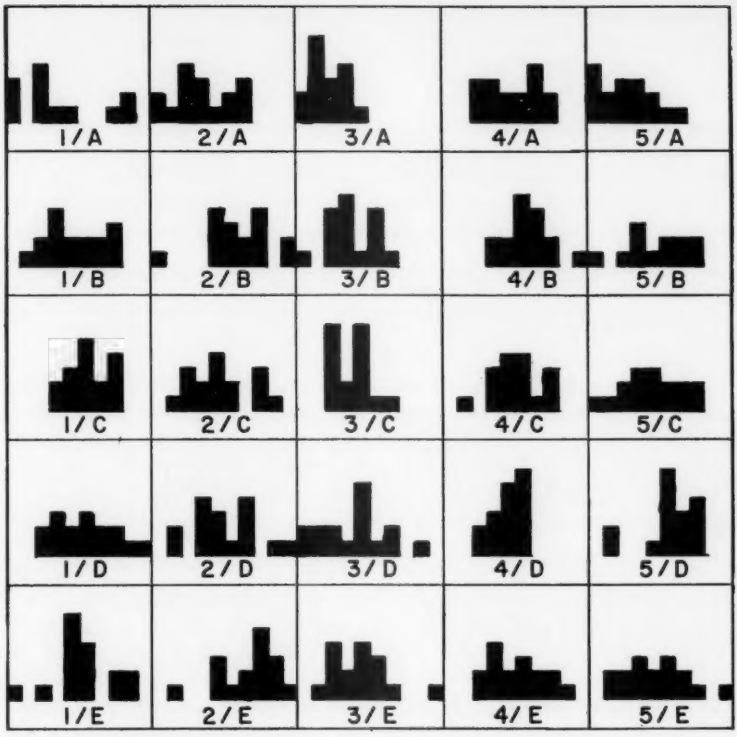


FIGURE 6.—Number of bristles on the 6th abdominal sternite of females homozygous for recombinant chromosomes recovered from females heterozygous for the 25 possible combinations of the 5 chromosomes from each two populations. Vertical and horizontal axes as in figure 5.

plished statistician. Second, the inclusion of undetectable non-crossovers among the chromosomes tested interferes with some formal assumptions. Third, the simple presentation of the qualitative analysis of the preceding section served to introduce the material available. It is a pleasure to acknowledge here the aid of DR. HOWARD LEVENE of the Department of Mathematical Statistics, Columbia University. The techniques for determining the contributions and interactions through an analysis of variance were supplied by him. Needless to say, sins of either omission or commission in the non-professional presentation of the mathematical procedures are those of the authors.

Technique

The statistical analysis can follow any one of several models; the results of the homozygous combinations can be included or excluded, the intra-popula-

tion combinations can be analyzed together with or separately from the inter-population combinations, and the two populations, likewise, can be analyzed together or separately. Not all of these analyses will be attempted here; we will study the populations separately keeping the intra-population analysis separate from that of the inter-population. The calculations will be made both including and excluding the homozygous combinations.

The statistical techniques and symbols will be explained using as examples the data on the viability of non-lethals. In order to keep the procedure manageable, the following approximations have been made: (1) In the case of cultures giving less than 50 flies, the number of wild flies observed has been increased to correspond to an observation based on 50 flies; a culture that gave 4 wild flies in a total of 21, for instance, has been regarded as having 9.5 wild flies.

TABLE 4

The behavior of the a/a female sterility in recombination. Fertile (+) and sterile (–) recombinants recovered from different females (I, II, etc.) carrying various chromosomal combinations of a and others.

	I		II		III		IV		Total	
	+	–	+	–	+	–	+	–	+	–
a/a		....		....		....		....		....
a/b	3	3	6	0	4	2	2	4	15	9
a/c	1	5	4	2	3	3	3	3	11	13
a/d	4	2	4	2	5	1	1	5	14	10
a/e	2	4	1	3	1	2	1	5	5	14
1/a	4	2	0	6	4	2	5	1	13	11
2/a	4	2	2	4	3	3	2	4	11	13
3/a	4	2	4	2	5	1	3	3	16	8
4/a	3	3	5	1	3	3	3	3	14	10
5/a	3	3	4	2	2	4	0	6	9	15
	28	26	30	22	30	21	20	34	108	103

(2) In computing grand averages, the averages of all combinations have been used with equal weight even though some combinations are not represented by 60 tested chromosomes.

Intra-population analysis, homozygous combinations included. In this analysis, as in all that follow, we assume that a certain mean viability (the mean number of wild flies in a sample of fifty), $\bar{x}$, is characteristic of homozygous recombinants of a given statistical population. Furthermore, we assume that the individual chromosomes tested (*1, 2, 3, ... m*, where *m*, equals *5*) make contributions to this mean viability that can be measured relative to one another ($a_1, a_2, \ldots a_m$ where Σa_i equals 0). Finally, the average viability observed for recombinants of a certain combination (x_{ij}.) is assumed to represent the sum of $\bar{x}$ (the population average) a_i and a_j (the contributions of the two chromosomes of the combination), and g_{ij} (a specific interaction of i with j). Actually, we have allowed for specific interaction and so this is not really an assumption but merely a way of expressing the true situation.

The contribution of a chromosome (1, for instance) to viability can be calculated as follows:

$$a_1 = \frac{2x_{11} + x_{12} + x_{13} \ldots + x_{1m}}{m + 2} - \frac{m + 1}{m + 2}\bar{x}, \text{ or}$$

$$= \frac{2(x_{11} - \bar{x}) + (x_{12} - \bar{x}) + (x_{13} - \bar{x}) + \ldots + (x_{1m} - \bar{x})}{(m + 2)}$$

The interaction of any two chromosomes (i and j, for instance) can be calculated:

$$g_{ij} = x_{ij.} - a_i - a_j - \bar{x}$$

The significance of the quantities calculated can be tested by an analysis of variance. Sums of squares for error are obtained as $\Sigma\Sigma\Sigma(x_{ijk} - \Sigma_{ij.})^2$—the summation through all combinations of the sums of squares of each combination where x_{ijk} is any individual observation and $x_{ij.}$ is $(\Sigma x_{ijk})/n$, the average of the combination. This sum of squares has degrees of freedom corresponding to the sum of the degrees of freedom of each combination.

The sum of squares for interaction equals the sum of all $n_{ij}(g_{ij}^2)$ (including g_{ii} for homozygous combinations), where n_{ij} equals the number of tested chromosomes of the i/j combination. This sum of squares has $m(m-1)/2$ degrees of freedom.

The sum of squares for contributions equals the sum of all $n_{ij}(a_i + a_j)^2$ (including $a_i + a_i$ for homozygous combinations). This sum of squares has $(m-1)$ degrees of freedom.

The test of significance of the observed interactions or contributions is afforded by the F ratio—the appropriate mean square divided by the mean square of the error.

It has been mentioned that both the sums of contributions and the sums of interactions are equal to zero. The hypothesis tested by the calculations is that the contributions or interactions observed are equal and, consequently, are equal to zero in every case. A significant result indicates that the different chromosomes do not contribute equally to the viabilities or, in the case of interaction, that they do not interact in a predictable manner with one another in the determination of the observed viabilities.

Intra-population analysis, homozygous combinations excluded. The inclusion or omission of the homozygous P_1 combinations from the analysis of the data is a matter of personal preference. It can be argued, for instance, that the combinations *1/1, 2/2, ... i/i* do not differ fundamentally from the combinations *1/2, 1/3, ... i/j* in that they represent living flies from which recombinant chromosomes can be obtained for testing. On the other hand, the viabilities of the homozygotes were known at the time the chromosomes were chosen; it was known that none of the ten was lethal, it was known that *4, 5, d* and *e* were at least subvital, and it was known that the other six chromosomes were normal in viability. There was no way, though, of knowing what recombinants

these chromosomes would produce. The homozygous combinations, then, may be eliminated from the calculations merely to remove a segment of data about which something was known in advance.

The statistical technique here is similar to that above. To avoid confusion, since the calculated values will differ from those of the first section, the symbols have been changed slightly; the changes are: x to y, a to b, and g to h.

As before, y_{ijk} is the number of wild flies counted in any one culture of the i/j combination and $y_{ij.}$ is the average number observed in the n_{ij} replicas. The average of all $y_{ij.}$'s equals $\bar{y}$. The contribution of a chromosome (1, for instance) to viability can be calculated as follows:

$$b_1 = \frac{y_{12.} + y_{13.} + \ldots y_{1m.}}{m-2} - \frac{m-1}{m-2}\bar{y}$$

$$= \frac{(y_{12.} - \bar{y}) + (y_{13.} - \bar{y}) + \ldots + (y_{1m.} - \bar{y})}{m-2}$$

The interaction between chromosomes is, again:

$$h_{ij} = y_{ij.} - b_i - b_j - \bar{y}$$

Once more the sum of contributions and the sum of interactions equal zero.

The sum of squares for error is calculated as before except that homozygous combinations are excluded from the computations. The sum of squares for interaction equals the sum of all $n_{ij}(h_{ij}^2)$ excluding homozygotes. The sum of squares has $m(m-3)/2$ degrees of freedom. The sum of squares for contributions equals the sum of all $n_{ij}(b_i + b_j)^2$ and has $(m-1)$ degrees of freedom. Once more the tests of significance are made by means of the F ratio.

Inter-population combinations. The inter-population combinations offer a simpler analytical problem than those considered above. First, all combinations are heterozygous. Second, the various combinations fall on a checkerboard rather than a triangular arrangement. Third, the data obtained met the standards of 60 tested chromosomes per combination, fifty flies sampled per chromosome. Hence the standard procedures of the analysis of variance may be used. These procedures are summarized below for the non-statistical reader.

The analysis is again based on the same model: the average number of wild flies observed in the recombinants of any combination can be analyzed in terms of the average number in the entire series, the contributions of the two chromosomes of the combination, and the specific interaction between the two chromosomes. Once more the symbols are changed slightly since the numerical values will differ from those obtained previously: x becomes z, a becomes c for population 1 and d for population 3, and g becomes e. The grand average, $\bar{z}$, equals $\Sigma z_{ij.}/m^2$. The contribution of a chromosome (1, for instance, equals $[(z_{1a.} + z_{1b.} + \ldots + z_{1e.})/5] - \bar{z}$. As before, interaction, e_{ij}, equals $z_{ij.} - c_i - d_j - \bar{z}$.

The sum of squares for error is computed just as in the two previous cases. Sum of squares for interaction equals the sum of all $n_{ij}(e_{ij}^2)$ and has $(m-1)^2$

degrees of freedom. The sum of squares for contributions of chromosomes from population 1 equals $\sum_i(\sum_j n_{ij}c_i^2)$; for chromosomes from population 3, $\sum_j(\sum_i n_{ij}d_j^2)$. Each of these sums of squares has $(m-1)$ degrees of freedom.

The analysis of contributions and interactions of chromosomes to bristle numbers is made by the same technique and with the same assumptions as the analysis of the viability data. The determination of error is changed slightly. Theoretically, all of the recombinants obtained from a homozygous combination should be as similar as the various individuals developing within any one culture. The large variance found between the four vials of any homozygous combination relative to the variance found within vials indicates that the culture conditions are contributing to variation in bristle numbers. Consequently, the error variance is based on the variation between vials of homozygous combinations. The analysis proceeds otherwise as in the analysis of viability where homozygous combinations were excluded.

Finally, the data on lethal frequencies can be treated in the same way as viability or bristle data although the figures are small. Some of the results of the lethal analysis are possible in theory only since there cannot be a frequency of lethals less than zero. Nevertheless, the results have meaning in connection with the other data as will be seen below.

Repeatability

A problem of immediate concern involves the repeatability with which recombinant types arise from a given combination of chromosomes. The number of gene combinations that can be formed by crossing over between two chromosomes is determined by the number of loci bearing contrasting alleles and the number of chiasmata at which exchanges take place. It has been estimated by IVES (1945) and WALLACE (1950) that on the second chromosome of *D. melanogaster* there are about 500 loci that can mutate to lethality; i.e., there is an observed probability of about .002 that any two independently arising lethals on this chromosome will behave as alleles. If two chromosomes were to differ in the alleles found at 500 loci, single crossovers could give rise to nearly 1,000 different combinations; double crossovers, theoretically, could produce some 250,000 more. It might be expected then that a study of a few crossover strands recovered from two chromosomes would be entirely inadequate to represent the array of possible combinations and that a second sample, through chance alone, might consist of an entirely different array. Different pairs of chromosomes producing seemingly unrelated arrays of crossover products may illustrate the improbability of recovering comparable samples rather than genetic differences between the chromosomes of the different pairs. The solution to this problem lies in the consistency with which different females carrying the same chromosome pair produce a given type or array of recombinants. If thousands of different gene combinations are possible and if each combination is unique, then different females of the same genotype would produce recombinants rivalling in variability those produced by females of different genotypes.

The material presented in tables 1 and 2 proves that such an extreme intra-combination variability does not occur; the evidence is striking even though the numbers of recombinants studied per female were relatively small. There is an obvious similarity in the frequency of lethals produced by the different females of the same genotype and, also, in the range of viabilities of non-lethals produced. In the case of lethals, the F ratio of the total variance and the variance between different females of the same genotype is 4.8; this ratio is significant at the .001 level. In the case of non-lethals, a comparison of the variances between means of different females and between recombinants of individual females for each genotypic combination revealed only four cases in which the variability found between means was greater than that expected from the variability between recombinants produced by the same female. Two of these instances were significant only at the 5% level (*3/5* and *5/e*); a third was significant at the 2.5% level (*1/5*); and a fourth was significant at the 0.5% level (*3/b*). Since an analysis of 45 cases should show by chance alone 2 cases seemingly significant at the 5% level and 1 at the 2.5% level, it seems likely that only the fourth case, *3/b,* has any real meaning. In this instance, one of the P_1 females (*3/b* III) gave rise to recombinants with consistently lower viabilities than did the other three; this could be comparable to the cases already mentioned in which certain females carried lethals of pre-P_1 origin. An extension of the analysis to all the data with each chromosomal combination treated as a group gives an F-ratio between variance of means and variance within groups of 18.1. This is highly significant. It appears that a given chromosomal combination possesses a limited number of divergent recombinant types. This number is small enough for sub-samples to show a correlation. A small number of recombinant types implies, in turn, a relatively low number of effectively recombining elements within any particular combination.

Analytical results

The results of the statistical analyses are listed in tables 5, 6, 7 and 8. Each table gives the relative contributions of the chromosomes to the trait under consideration, the specific interactions of different chromosomal combinations, and the statistical significance of the data. In one instance (table 5b) the tests of significance have been omitted because the data were incomplete.

In their contributions to viability (tables 5, 6 and 7) the chromosomes differ significantly from one another and, with minor exceptions, make the same relative contributions whether homozygous combinations are included or excluded from the calculations or whether the heterozygous combinations are intra- or inter-population. The one apparent difference is an increase in the contribution of chromosome *c* in inter-population combinations compared to its contribution in intra-population #3 combinations. The differences in contributions of all chromosomes is highly significant ($p < .001$) but it must be remembered that four of the original chromosomes were chosen for poor viability.

In the combinations in which significance was tested (tables 5, 6 and 7) the interactions of the specific chromosome pairs differed significantly. The test

fails to reveal the specific pairs that cause the heterogeneity but it might be assumed that the ones with the largest interactions (ignoring signs) are the most likely ones. In that case, the combination *4/5* is interesting. These two chromosomes gave recombinations at least one half of which were lethal. In addition, we now see that recombination of these chromosomes produced non-lethals of lower viabilities than one would expect from their individual contributions in other combinations. Aside from this case there are about a dozen other combinations that gave seemingly significant interactions: *a/b, a/d, a/e, b/c, c/d, c/e, 1/d, 3/b, 3/d, 3/e, 4/b, 4/e* and *5/a* to indicate the more probable ones. One can safely conclude that, in determining the viability of homozygous crossover chromosomes, specific genic interactions occur that result in recombinants of unexpected but characteristic viabilities for the different chromosome pairs.

TABLE 5

Interactions and contributions of chromosomes to the viability of non-lethal recombinants.

Intra-population #1, homozygous combinations included							
a_1	0.48	g_{11}	−0.12	g_{12}	−0.28	g_{34}	−0.11
a_2	0.31	g_{12}	−1.00	g_{23}	−0.34	g_{35}	1.52
a_3	2.76	g_{13}	0	g_{24}	0.36	g_{44}	0.24
a_4	−0.86	g_{14}	−0.04	g_{25}	1.59	g_{45}	−0.64
a_5	−2.69	g_{15}	1.29	g_{33}	−0.54	g_{55}	−1.87

	ss	df	Mean square	F-ratio	p
Error	14799.98	868	17.05	....	....
Interaction	704.6330	10	70.4633	4.13	<.001
Contribution	6470.4631	4	1617.6158	94.87	<.001

Intra-population #3, homozygous combinations included*							
a_a	0.66	g_{aa}	−1.28	g_{bb}	−0.47	g_{cd}	−1.30
a_b	2.04	g_{ab}	−0.30	g_{bc}	0.59	g_{ce}	0.44
a_c	1.59	g_{ac}	−0.90	g_{bd}	0.60	g_{dd}	−1.99
a_d	−1.70	g_{ad}	4.18	g_{be}	0.05	g_{de}	0.54
a_e	−2.59	g_{ae}	−2.59	g_{cc}	0.60	g_{ee}	−0.24

*Assuming that $x_{aa} = \bar{x}$, a_a and g_{aa} obtained through equations $\Sigma a_i = 0$ and $\Sigma g_{ij} = 0$. No tests of significance were made.

(Two precautionary notes should be inserted here. First, our " contributions " and " interactions " have only a superficial resemblance to the " general combining ability " and " specific combining ability " of hybrid crosses with which most readers are familiar. The final (F_3) crosses of this experiment produced flies homozygous for certain recombined second chromosomes so inter-allelic interactions—dominance and over-dominance—were eliminated; instead, these tests reveal inter-locus epistatic interactions. Second, the patterns of viabilities as shown in figure 3 do not necessarily reflect in any orderly way the types of recombinations of the two chromosomes. Chromosomes possessing the same viability may arise from entirely different crossover patterns within a combination while others possessing different viabilities may differ by only the slightest shift in the position of a chiasma. There is no way by

TABLE 6

Interactions and contributions of chromosomes to viability of nonlethal recombinants.

Intra-population #1, homozygous combinations excluded					
b_1	0.21	h_{12}	−0.84	h_{24}	0.74
b_2	0.16	h_{13}	0	h_{25}	0.58
b_3	2.77	h_{14}	0.46	h_{34}	0.11
b_4	−1.35	h_{15}	0.40	h_{35}	0.35
b_5	−1.79	h_{23}	−0.46	h_{45}	−1.31

	ss	df	Mean square	F-ratio	p
Error	10705.61	589	18.1759	....	....
Interaction	239.6939	5	47.9388	2.64	<.05
Contribution	2288.5964	4	572.1491	31.49	<.001

Intra-population #3, homozygous combinations excluded					
b_a	1.03	h_{ab}	−0.87	h_{bd}	−0.49
b_b	1.90	h_{ac}	−0.77	h_{be}	0.13
b_c	0.75	h_{ad}	2.58	h_{cd}	−1.69
b_d	−0.81	h_{ae}	−0.95	h_{ce}	1.22
b_e	−2.87	h_{bc}	1.23	h_{de}	−0.41

	ss	df	Mean square	F-ratio	p
Error	13600.62	590	23.05	....	....
Interaction	911.4720	5	182.2944	7.91	<.005
Contribution	2542.7520	4	635.6880	27.58	<.001

which these two phenomena—chiasma position and resultant viability—can be related in this experiment; this is unfortunate because they may not be as haphazardly related as may be imagined.)

The bristle analysis revealed a situation different from that indicated by the viability studies. There were no statistically significant interactions in either intra- or inter-population combinations in the determination of bristle numbers.

TABLE 7

Interactions and contributions of chromosomes to the viability of inter-population recombinants.

		c_1	0.32			d_a	1.23		
		c_2	0.22			d_b	2.02		
		c_3	1.79			d_c	1.89		
		c_4	−0.79			d_d	−1.84		
		c_5	−1.53			d_e	−3.29		
e_{1a}	−0.56	e_{2a}	0.44	e_{3a}	0.77	e_{4a}	0.28	e_{5a}	−0.96
e_{1b}	−0.12	e_{2b}	−0.68	e_{3b}	−1.09	e_{4b}	1.13	e_{5b}	0.77
e_{1c}	−0.34	e_{2c}	0.06	e_{3c}	−0.21	e_{4c}	−0.04	e_{5c}	0.51
e_{1d}	0.83	e_{2d}	0.31	e_{3d}	−1.53	e_{4d}	0.42	e_{5d}	−0.06
e_{1e}	0.16	e_{2e}	−0.16	e_{3e}	2.04	e_{4e}	−1.78	e_{5e}	−0.29

	ss	df	Mean square	F-ratio	p
Error	29700.30	1475	20.14	....	....
Interaction	1009.8600	16	63.12	3.13	<.005
Contribution (1)	1895.9700	4	473.9925	23.53	<.001
Contribution (3)	7012.5300	4	1753.1325	87.05	<.001

TABLE 8

Interactions and contributions of chromosomes to bristle numbers of homozygous recombinants.

Intra-population #1

b_1	1.33			h_{12}	−0.10	h_{34}	0.34		
b_2	5.33			h_{13}	2.15	h_{35}	0.84		
b_3	−5.92			h_{14}	−2.41	h_{24}	1.09		
b_4	−0.61			h_{15}	0.34	h_{25}	−2.16		
b_5	−0.11			h_{23}	−1.10	h_{45}	0.95		

	ss	df	Mean square	F-ratio	p
Error	342.01	18	19.01	....	....
Interaction	76.4719	5	15.2944	<1	>.5
Contribution	786.7840	4	196.6960	10.35	<.001

Intra-population #3

b_a	−2.40	h_{ab}	1.35	h_{bd}	−0.26		
b_b	−0.04	h_{ac}	−0.79	h_{be}	−1.01		
b_c	3.27	h_{ad}	−0.40	h_{cd}	0.18		
b_d	−0.04	h_{ae}	−0.15	h_{ce}	0.68		
b_e	−0.79	h_{bc}	−0.07	h_{de}	0.95		

	ss	df	Mean square	F-ratio	p
Error	342.01	18	19.01	....	....
Interaction	17.2023	5	3.4405	<1	>.5
Contribution	204.2055	4	51.0514	2.69	>.05

Inter-population

c_1	1.28	d_a	−5.17	e_{1a}	−0.04	e_{3a}	−1.48	e_{5a}	−1.53
c_2	2.30	d_b	1.70	e_{1b}	−2.58	e_{3b}	−0.60	e_{5b}	−0.40
c_3	−4.45	d_c	0.35	e_{1c}	2.52	e_{3c}	0.75	e_{5c}	−0.55
c_4	1.75	d_d	0.95	e_{1d}	1.17	e_{3d}	0.40	e_{5d}	3.85
c_5	−0.90	d_e	2.15	e_{1e}	−1.03	e_{3e}	0.95	e_{5e}	−1.35
				e_{2a}	−1.48	e_{4a}	4.57		
				e_{2b}	1.90	e_{4b}	1.70		
				e_{2c}	−2.25	e_{4c}	−0.45		
				e_{2d}	−1.85	e_{4d}	−3.55		
				e_{2e}	3.70	e_{4e}	−2.25		

	ss	df	Mean square	F-ratio	p
Error	342.01	18	19.01	....	....
Interaction	434.5576	16	27.1599	1.43	>.05
Contribution (1)	609.6196	4	152.4049	8.02	<.005
Contribution (3)	675.7091	4	168.9273	8.89	<.005

There is no good evidence, in fact, that the different chromosomes of population #3 made different contributions to the bristle numbers of intra-population combinations. The situation is complicated, however, because the contributions of chromosomes of population 3 changed sharply between intra- and inter-population combinations. The contributions of *a*, *c* and *e*, for instance, were −2.40, 3.27 and −0.79, respectively, in intra-population combinations. In inter-population combinations these values became −5.17, 0.35 and 2.15—changes of −2.77, −2.92 and 2.94. These changes, apparently, were those that made the contributions of chromosomes from this population significantly different in

the inter-population combinations. An absence of demonstrable interactions in both intra- and inter-population combinations does not indicate such genetic uniformity that chromosomal contributions within the two systems must necessarily be equal.

These results show that two polygenic characters—viability and abdominal bristles—need not reflect the past history of a population by possessing identical reaction patterns. In the case of viability, the different chromosomes made characteristic contributions whether in intra- or inter-population combinations and there were numerous instances of statistically significant interactions between members of various pairs; in the case of abdominal bristles, significantly different contributions were found in one population only and there were no cases of significant interactions. It should be noted, however, that the residual error variance was large for bristles; an analysis of greater numbers of flies for this trait may have revealed significant interactions. In the bristle analysis large differences in the contributions of chromosomes between intra- and inter-population combinations were found.

Differentiated genetic systems

The possibility was mentioned above that evidence for coadaptation might be obtained if the formation of ill-adapted complexes by recombination among inter-population combinations was observed. The evidence for differentiated genetic systems is extremely subtle; nowhere in the data does mere inspection reveal interactions comparable to that shown in figure 1 where nearly every recombination behaved as a lethal.

In spite of an apparent similarity there is one detectable difference in the behavior of chromosomes in intra- and inter-population combination. This difference lies in the patterns of contributions. The contributions of the ten chromosomes to lethality, viability, and bristle numbers have been consolidated in table 9. Taking the data at face value and considering only signs, there is a perfect negative correlation between contributions to lethality and viability

TABLE 9

The contributions of the different chromosomes to lethality, viability, and bristles of flies homozygous for recombinations of intra- and inter-population heterozygous combinations.

Chromosome	Intra-population			Inter-population		
	Lethal	Viab.	Bristle	Lethal	Viab.	Bristle
1	−0.054	0.21	1.33	0.002	0.32	1.28
2	−0.060	0.16	5.33	−0.015	0.22	2.30
3	−0.057	2.77	−5.92	0.001	1.79	−4.45
4	0.084	−1.35	−0.61	0.004	−0.79	1.75
5	0.087	−1.79	−0.11	0.009	−1.53	−0.90
a	−0.010	1.03	−2.40	−0.012	1.23	−5.17
b	−0.010	1.90	−0.04	0.012	2.02	1.70
c	−0.026	0.75	3.27	−0.001	1.89	0.35
d	0.009	−0.81	−0.04	−0.013	−1.84	0.95
e	0.037	−2.87	−0.79	0.014	−3.29	2.15

among the intra-population combinations (p about .002). Every chromosome that tended to reduce viability tended also to increase the frequency of lethals among its recombinants; every chromosome that tended to increase viability tended to lower the frequency of lethals. Within the inter-population combinations there is no comparable correlation; contributions to lethality and viability of non-lethals are seemingly independent of one another.

The contrast between the patterns of contributions of chromosomes in intra- and inter-population combinations is apparently the result of lethal recombinations. The signs of the contributions of the various chromosomes to viability in the two types of combinations also show a perfect correlation as has been pointed out above. The contributions to lethality vary considerably from one type of combination to the other just as the contributions to bristle numbers did. How much of the perfect correlation of the signs of the viability contributions is the result of undetectable non-crossovers is unknown, of course. The contributions of the chromosomes to bristle numbers are also listed in table 9; these contributions are seemingly unrelated to the others in both types of combinations.

The contrasting patterns of contributions described suggest different genetic components within the gene pools of the two populations. Since natural selection operates to produce the highest possible adaptive value in each population, the differences between the gene pools reflect at least some degree of coadaptation within one or both of the populations.

CONCLUSIONS

The aim of the experiments described above was to decide which of two possible genetic systems natural selection tends to establish within a population. One hypothesis postulated a genetically homogeneous system—every locus represented for the most part by a single "wild type" allele (A, B, C, . . .) or, to the small extent that mutation operates counter to selection, a few recessive mutant alleles (a, b, c, . . .). The second hypothesis, rejecting the assumption that homozygosity leads to the highest adaptive value, postulated a genetically heterogeneous system—each locus represented by a series of multiple alleles selected on the basis of the alleles at all other loci and the adaptive values of their many possible combinations.

Before discussing the results, some of the assumptions on which this experiment was based must be examined. First, we have assumed that chromosomes with new phenotypic properties derived from certain chromosomal combinations are crossover chromosomes. Second, we have assumed that the altered phenotypic effects result from new combinations of the various loci. The physical distances between these loci, in any case, are unknown. Third, we have assumed that a representative sample of crossover chromosomes is recovered for analysis. There is no *proof* that these assumptions are correct. That crossing over generally occurs in female Drosophila can be demonstrated by the use of marked chromosomes; there is no obvious reason why it should not also occur in the absence of mutant genes. We know, with reference to the second

point, that mutations occur and that their rates vary but a rate of mutation to lethals of 43% (as in *4/5*) or to a single visible of 10% (as in *d/e*) is higher than even the mutable stocks of IVES (1950). MISRO (1949) found that high frequencies of lethals were produced characteristically only by females heterozygous for different second chromosomes; heterozygous males, with their lack of recombination, produced lethals at the same low rate as homozygous females. The ten chromosomes of this analysis were examined cytologically and no aberrations were found that would interfere with "free" crossing over. Just how "free" crossing over is between pairs of different chromosomes is a moot point; the frequency of recombination of mutant marker genes carried by different females is extremely variable as GOWEN (1919) has shown. Partial localization of chiasmata between pairs of different chromosomes may account in part for the varying arrays of recombinants obtained. We will assume, therefore, that the experimental results are to be explained by the shuffling of chromosomal elements but whether the physical act of shuffling within one combination is similar to that of all others is unknown.

Some of the experimental results are consistent with the assumption of homogeneity within the genetic systems of the populations studied. First, there is the correlation between lethal frequencies (or viability arrays of non-lethals) from different females of the same genotype; the intra-combination variance was much smaller than the inter-combination variance. This suggested that the chromosomes of the various pairs differed in only a few essential elements and that the number of different recombinants per combination was limited. Second, the sterility factor of chromosome *a* behaved as a simple Mendelian unit in all combinations with no detectable interactions.

Much more of the information presented tends to support the second hypothesis—that of genetic heterogeneity. Especially important in this respect are the combinations *4/5* and *d/e* and the other combinations in which these four chromosomes participated.

Of the crossover chromosomes obtained from the *4/5* combination, 43% were lethal—48 lethals in 112 tests. The 95% confidence interval of this frequency extends from 33% to 53%. Within this interval fall the results predicted by two simple hypotheses: (a) that there are two complementary genes, widely separated, of which chromosomes *4* and *5* represent the only viable combinations; and b) that there is multigene complex such that one half of all crossovers are lethal. The first scheme would result in 50% lethals among the chromosomes tested and the second in about 35% (50% of the 70% real crossovers). The two-gene hypothesis requires an elaboration for not only must chromosomes *4* and *5* differ from one another but these chromosomes must also differ from all the other chromosomes in the alleles at these two loci. At each of two loci, then, there must be a series of three alleles—a, a′, a″, b, b′ and b″—revealed by a test of five chromosomes of population 1. This qualification is necessary because neither *4* nor *5* produced lethals in combination with any other chromosome of population 1. The hypothesis of a multigene complex does not require the postulation of a series of multiple alleles but it

requires more loci. This hypothesis meets with the difficulty that the viability of non-lethal recombinations is not improved as a result of the loss of certain genes in the lethal chromosomes. Therefore, the elements that enter into the lethal complex must have no detectable effect on viability in the rest of the possible combinations of these two chromosomes.

It is tempting to ascribe the lethal-forming capacity of the *4/5* combination to a subvital on *4* and a semilethal on *5*. If the genetic elements responsible for these characteristic viabilities of the two chromosomes are involved, the interaction must be quite specific. Chromosome *e*, a semilethal, gives 9% lethals through recombination with chromosome *4*. If one imagines that alleles at specific loci are recombining to give the 43% lethals from the *4/5* combination and the 9% from the *4/e* combination, then one would imagine that there would be ample opportunity for *5/e* lethal recombinations to occur. Contrary to expectation, only 1% of these recombinations are lethal. This requires the postulation of more contrasting alleles to account for the *4/e* lethals and the lack of *5/e* lethals. Then more alleles are needed to explain why most *3/5* recombinants are like those of *3/3* while many from *1/5* are like those of *5/5*. It appears that a sample of five chromosomes taken from a population contains a substantial number either of demonstrable multiple allelic series or of interacting mutant alleles at a variety of loci.

It was mentioned earlier that ten percent of the recombinants of *d* and *e* produced a visibly abnormal eye condition. Once more, to explain the absence of this type of eye in *d/d, e/e* and all homozygous recombinations of *d* and *e* with other chromosomes, one must postulate at least two genes with contrasting alleles on *d* and *e* and a still different allele on all other chromosomes. Then, since 10% of the recombinations of this pair were also lethal, a similar series must be postulated for this trait. (An apparent simplification may be made by assuming that in certain combinations the lethal complex becomes viable but results in a modification of the eye.) More and more complications arise when one tries to account for the variety of lethal frequencies that result from various combinations involving *d* and *e;* in some corresponding combinations (e.g., *a/d* and *a/e*) the sum of the two frequencies is nearly equal to the 10% of *d/e,* in other such combinations there is no relation between these results and that of *d/e*.

These detailed examinations of chromosomes *4, 5, d* and *e* in combination with one another lead to complex schemes of multiple alleles reminiscent of the heterogeneous genetic system outlined in the original argument. Repeatedly one is forced to create new allelic series or new interacting mutant genes at many loci. In many instances, these hypothetical mutants have no effect other than the one that led to their postulation.

If these data fit the hypothesis of genetic heterogeneity so well, how is one to account for that evidence consistent with the concept of homogeneity?

If two populations were started with comparable samples of chromosomes (as 1 and 3 were) and an experiment of the sort reported here were made during the second generation, one would expect to find that the chromosomes

of the two populations were still mostly alike. Between this time of intra- and inter-population uniformity and the formation of coadapted systems such as are found in natural populations of long standing, there must be a gradual transition; 60 generations may well represent an intermediate time at which conflicting results can be obtained.

It was seen above that some of our postulated genetic variation became apparent only in certain combinations. It was also noted previously that the statistical contributions of chromosomes to bristles and to lethals were quite different in intra- and inter-population combinations. It is entirely possible that the effects of some series of alleles or of certain interacting complexes become apparent only in the new combinations of inter-population complexes. Intra-population combinations may allow these complexes to remain cryptic and, hence, to exaggerate the appearance of uniformity.

Finally, the inclusion of non-crossovers among the chromosomes designated " recombinants " would have the effect of lowering intra-combination variance. In the case of lethals—which *must* be crossovers—certain females with aberrant lethal frequencies were eliminated from the data. The first of these procedures is unavoidable, the second is probably correct; both tend to increase the calculated homogeneity of certain combinations, however, and thereby detract from the force of arguments based on this homogeneity.

With a great deal of patience models of the ten chromosomes could undoubtedly be made that would explain these results. Whether or not such models could actually be made is not important. What is important is the interdependence between the postulated alleles and the number of loci. There are certain anomalies to be explained in various combinations and these explanations will require either alleles with new properties or combinations in which given alleles acquire novel characteristics. Furthermore, the novel attributes of new combinations can completely submerge the previous properties of the elements involved. Is there a lethal gene on chromosome *3?* or *c?* These chromosomes are the two most viable of the ten; still 5% of their recombinations are lethal. These two points—the existence of interactions and the completely different phenotypic roles played by the assorting parts of interacting complexes as they are shuffled and reshuffled—are the core of the second hypothesis. Selection is acting in these populations to build heterogeneous genetic systems.

DISCUSSION

Genes are often thought of as representatives in the germ plasm of certain phenotypic traits. This is apparent in the names given to genes—a lethal, a white-eye or a frizzled-feather gene. Despite protestations to the contrary, this naïve concept occurs even among geneticists; KEMP (1951, p. 31), for instance, says that a gene " determines a character in the adult organism." A majority of geneticists are not interested primarily in the physical dimensions of a gene or in the existence of sub-genes demonstrable by refined techniques; rather, they are interested in the " names " of genes, in the spectacular array of

mutant varieties, in the light shed by these abnormalities on physiology and development, in the practical utilization of mutant characters, and in the role these mutant genes play in populations.

Furthermore, many biologists still think of populations in terms of the type-species concept; a population is imagined to be a collection of genetically uniform individuals. The " pure race " theories, it is true, have fallen into disrepute but the thinking of some geneticists still continues to be colored by these theories. The genetic variation within populations is imagined to be caused by sharply defined mutant alleles at a relatively small number of loci; races are characterized by the differing frequencies of these mutants. Aside from this variable part of the genotype, the species is supposed to be essentially homozygous for a large reservoir of genes—sometimes referred to as " species genes." That a rigid adherence to these two concepts results in unnecessarily complicated genetic interpretations is attested by the conclusion—in human genetics—that most mutant genes are semidominant with incomplete penetrance. The admission of genic interaction and genetic variability leads one to expect the complicated inheritance patterns observable in human populations.

It is not implied here that the expressions of all genes are so fluid that naming them according to their most striking phenotypic characteristics is unjustified. It is common knowledge that many genes express themselves in an extremely large number of genetic backgrounds. For example, white-eye, forked, scarlet, or the *a/a* female sterility of this study—each produces approximately similar phenotypes with nearly all genetic backgrounds tested. It is not so well known, however, that " good " genes are a relatively small fraction of the total number of hereditary variants known. In analyzing the genetic system of a population in terms of " fitness " or adaptive value, the most accurate and most convenient concept is not necessarily one derived from culture-bottle pure-line techniques. Such a concept, even when modifications are admitted, tends to minimize the frequency and importance of genic interactions.

This argument involves more than terminology. If the gene is labeled then even if it fails to " penetrate," one imagines the mutant allele acting in accordance with its label. Under the other view, which the data here clearly support, there is scarcely any place to attach a label; the phenotypic role of a segment of a chromosome depends upon the particular genetic combination in which it is operating. This role can vary considerably from combination to combination. Labelling genes leads to problems concerning static quantities: How many loci have heterotic alleles? How semidominant is a deleterious gene? How many loci can mutate to lethality? What is the selective value of a given mutant? The latter concept alters these questions; the new emphasis is on probability or frequency: What is the probability that a given allele will be heterotic in any one of a variety of combinations? What is the mean selective value of a gene in a population? What is its variance? Can selection act to consolidate favorable gene complexes? At what rate? Failure to appreciate the frequency and magnitude of specific interactions can easily lead to conclusions that have little bearing on natural systems.

SUMMARY

Two hypotheses dealing with the genetic structure of populations have been presented together with a technique for discriminating between them. One hypothesis states that selection within a population favors a particular allele at each locus and that, as a result, all individuals have essentially the same genotypes. The second hypothesis states that selection favors particular heterozygotes and thereby builds up selected series of alleles at all (or at least many) loci—the particular alleles at any locus being determined in each population by those present at all other loci. The technique for discriminating between these hypotheses is based on the types of recombinant chromosomes recoverable from different combinations of chromosomes isolated from two different populations.

Five non-lethal second chromosomes were chosen from each of two experimental populations of *D. melanogaster,* and females carrying all 55 possible combinations of these chromosomes were made by intercrossing balanced stocks. Crossover chromosomes were recovered from each of these combinations and these were made homozygous. The homozygous recombinants were then examined for lethality, for their effects on viability if non-lethal, for their effects on abdominal bristle number, and for their effects on a particular female sterility factor included by chance in the original material.

The experimental results favor the second hypothesis for the following reasons:

1) The two members of pairs of chromosomes that produce recombinants highly similar in viability frequently produce entirely different arrays when in combination with a third chromosome.

2) Specific pairs of chromosomes produce frequencies of lethals unlike those that either produced in other combinations. These cases of specific interaction call for the postulation of multiple alleles or interacting series of mutant genes undetectable in other combinations.

3) An analysis of variance reveals specific contributions of chromosomes and specific interactions of chromosome pairs in determining viability of crossovers.

The contrasting impacts of the two hypotheses on our thinking of population problems have been discussed. The question is raised whether, in the face of complex genetic structures and the numerous instances of gene interactions, the concept of a "phenotypic" gene derived from studies on homogeneous experimental material is a useful one for the understanding of populations. It is suggested that more information concerning the complications possible within populations is needed; without this information any concept of population dynamics risks being spuriously simple.

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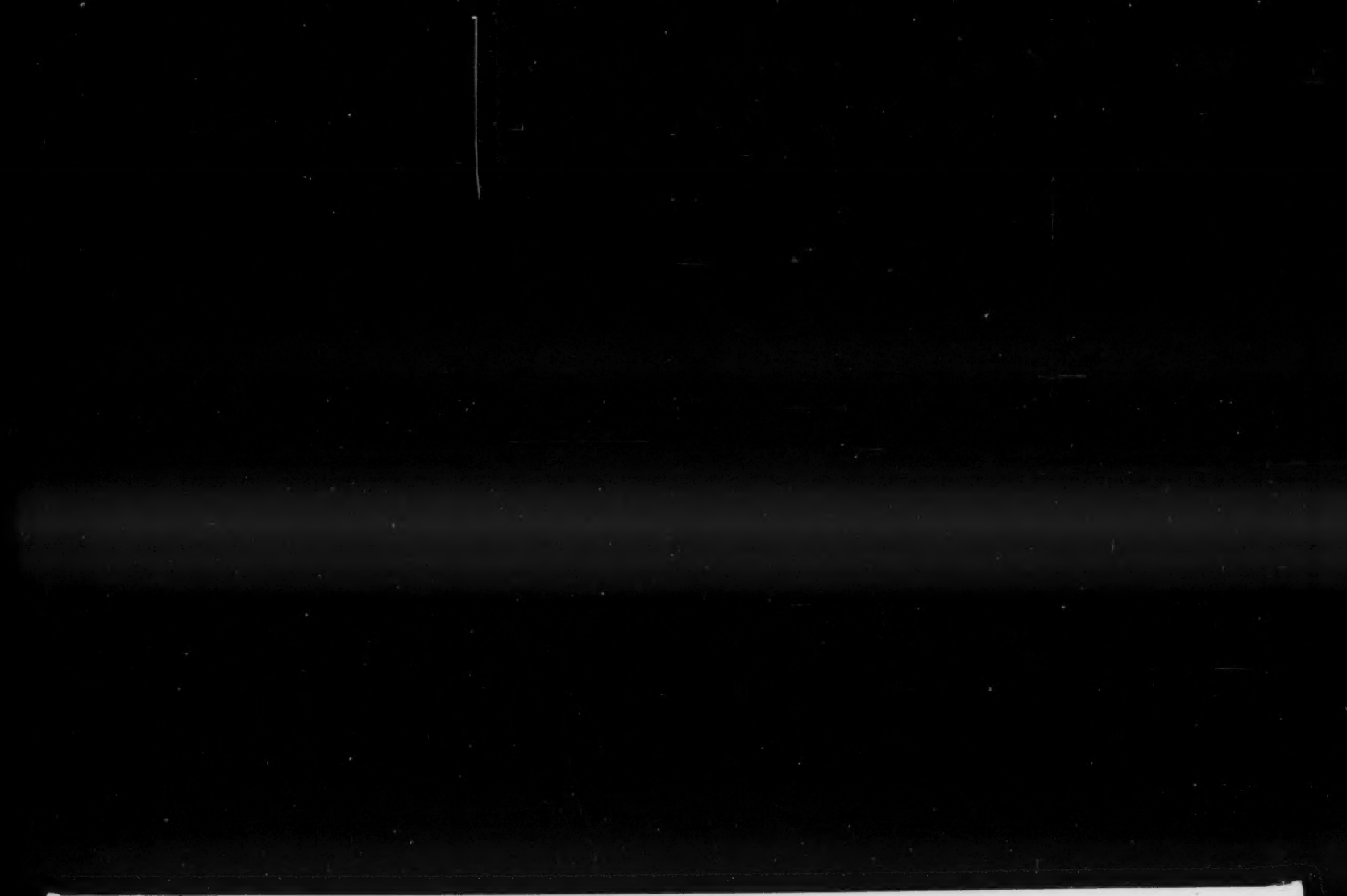
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